This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

Stanford University Libraries
3 6105 006 872 035

LELAND·STANFORD·JVNIOR·VNIVERSITY

THE
AMERICAN JOURNAL
OF
PHYSIOLOGY

VOLUME XLVII

STANFORD LIBRARY

BALTIMORE, MD.
1918–1919

263100

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 SEPTEMBER 1, 1918 No. 1

THE ACTION OF PITUITARY EXTRACT ON THE KIDNEY

F. P. KNOWLTON AND A. C. SILVERMAN

From the Physiological Laboratory, College of Medicine, Syracuse University

Received for publication June 11, 1918

The essential contribution of this paper is a study of the changes in the blood flow and the oxygen consumption of the kidney during the diuresis evoked by pituitary extract. This was done in an endeavor to throw further light on the mode of action of the extract on the kidney, a question to which conflicting answers have been given. These contradictions result from the dual nature of the kidney mechanism and the complex circulatory conditions obtaining in this organ which make it difficult to explain results on the basis of vascular and volume changes.

Magnus and Schäfer (1) and Schäfer and Herring (2) were the first to recognize and investigate the diuretic action of extracts of the pituitary body. They explained the diuresis as due to direct stimulation of the renal epithelium. Their conclusion was based on the lack of parallel between the diuretic effect on the one hand and the blood pressure and kidney volume on the other hand. For a discussion of the data upon which this conclusion was based the reader is referred to Schäfer's recent review of the subject (3). According to his conception, the active diuretic principle bears the same relation to the kidney that secretin bears to the pancreas. Hoskins and Means (4) have supported Schäfer's view. An opposite conclusion has been arrived at by Houghton and Merrill (5) and by King and Stoland (6). From a comparison of the blood pressure, kidney volume and urine flow they consider that the vascular changes following the injection of pituitary extract are sufficient to explain the diuresis. Pal (7) and later Cow (8) working with isolated arterial rings, after the method of Meyer, found a specific dilator action on the renal vessels. McCord (9) found that the vessels of the isolated perfused kidney were dilated by pituitary extract while the vessels of other organs (e.g., spleen) were constricted.

In view of these conflicting opinions it seemed to the authors that a study of blood flow and oxygen consumption, using the method of Barcroft and Brodie (10), might throw further light on the question. Using this method, Barcroft and Straub (11) showed that under certain conditions (e.g., injection of Ringer's solution) well-marked diuresis may be induced without any change in oxygen consumption by the kidney. The diuresis induced by other means (e.g., urea injection) was accompanied by a decided increase in oxygen intake. It was considered therefore that, if the diuresis induced by pituitrin was associated with an increase of metabolic activity as indicated by the oxygen intake, this would furnish additional confirmation of Schäfer's view, while on the contrary if no change in metabolic activity was found, it would be reasonable to consider the process purely physical.

EXPERIMENTAL

The experiments were mainly on cats. The animals were anaesthetized with urethane supplemented by ether when necessary. The methods of dissection, collection of the blood and measurement of its rate of flow were similar to those previously described by Barcroft and Brodie (10). In a few experiments on pregnant cats, evisceration was avoided by using the greatly enlarged ovarian vein for the measurement of the blood flow. In this way the nerve supply to the kidney was preserved intact. This method was suggested to one of us by Mr. Barcroft. Urine was collected by means of a bladder cannula and the flow recorded by a drop-counter. Oxygen determinations were made with the Barcroft blood-gas apparatus, using the differential method described by Barcroft (12).

The preparations used were pituitrin (Parke, Davis & Company) and infundin (Burroughs Welcome). In experiments where the two preparations were used alternately a greater preliminary fall of blood pressure was noted with the infundin, indicating perhaps a slightly greater content of accompanying vasodilator substance. Otherwise the action of the two preparations appeared identical.

In general the results we obtained were in conformity with those of Schäfer and his coworkers. The intravenous injection of pituitary extract was commonly followed by an initial fall of blood pressure.

Apparently this fall was peripheral in origin rather than cardiac as it appeared as promptly when the injection was made directly into the arterial system after the method devised by Mathison (13). This brief

TABLE 1

*Effect of repeated injections of pituitary extract on blood pressure, urine flow and blood flow**

EXPERIMENT	INJECTION NUMBER	TIME INTERVAL	BLOOD PRESSURE (CAROTID)		URINE FLOW (DROPS PER MINUTE)		BLOOD FLOW (CUBIC CENTIMETERS PER MINUTE)		REMARKS
		Minimum	Before	After	Before	After	Before	After	
A	1		126	150–124	3	17			Infundin 0.25 cc. in 5 cc. saline
	2	30	110	120–100	11	16			Infundin 0.25 cc. in 5 cc. saline
	3	30	116	120	12	41			Infundin 0.25 cc. in 5 cc. saline
	4	30	108	120	11	34			Pituitrin, 0.5 cc.
	5	30	116	128	8	22			Pituitrin, 0.5 cc.
	6	30	118	120	12	17			Infundin, 0.25 cc.
B	1		162	186–174	12	24			Pituitrin, 0.5 cc. in 2 cc. saline
	2	15	165	178	18	27			Pituitrin, 0.5 cc. in 2 cc. saline
	3	15	160	172	14	24			Infundin, 0.25 cc. in 4 cc. saline
	4	15	140	186	14	24			Infundin, 0.25 cc. in 4 cc. saline
	5	15	140	148	9	13			Pituitrin, 0.5 cc.
	6	15	146	160	11	14			Infundin, 0.5 cc.
	7	15	144	148	8	14			Infundin, 0.5 cc.
	8	15	142	142	8	16			Pituitrin, 1 cc.
C	1		152	188	6	16	27.9	37.8	Pituitrin, 1 cc.
	2	15	152	164	4	27	29.2	48.5	Pituitrin, 1 cc.
	3	20	154	160	7	27	34.6	44.4	Pituitrin, 1 cc.
	4	20	142	142	16	30	44.4	55.8	Pituitrin, 1 cc.
	5	15	140	130	14	23	45.2	56.6	Pituitrin, 1 cc.
	6	20	120	120	7	17	44.5	46.5	Infundin, 0.5 cc.

* The measurements of blood pressure as given in the tables were taken just at the time when the blood flow was measured. They therefore do not always indicate the maximum point reached as a result of the injection. Our plan was to take the blood flow and samples for analysis at the point when urine flow seemed at its maximum.

initial fall was followed by a rise which persisted for a variable time. Associated with these circulatory changes there was first suppression or diminished secretion of urine coinciding with the fall of blood pressure. Diuresis as a rule began just beyond the crest of high pressure. Our observations also confirm the statement of Magnus and Schäfer, and Schäfer and Herring that the diuresis may outlast the rise of blood pressure or may be copious with a fall.

Repeated injections gave, in general, diminishing effects on the blood pressure so that after the first two or three injections the initial fall was the prominent feature and the after-rise often failed to reach the earlier average. With few exceptions the diuretic effect also tended to diminish with repeated dosage. This diminution of diuretic action was not as definite nor was it parallel with the lessening circulatory effect.

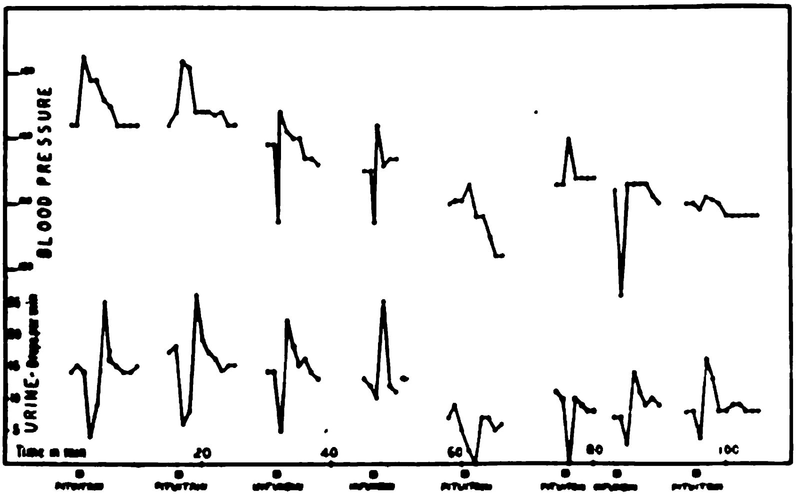


Fig. 1

By referring to table 1 the reader can compare the above described changes in detail. It may be seen that in experiments A and B the maximum rise of blood pressure followed the first injection while the maximum urine flow and blood flow through the kidney came subsequent to the second and third injections respectively. In experiment C the maximum blood pressure change and maximum urine flow approximately coincided. In figures 1 and 2 the results of repeated injections are presented graphically.

In table 2 the effects of pituitrin on blood pressure, blood flow through the kidney and diuresis are compared in greater detail. It is seen that whether the arterial blood pressure rises or falls, increased flow of blood through the kidney is a constant accompaniment of diuresis. In a general way the two effects run parallel, a free diuresis being associated with a decided increase in blood flow. In a number of instances the quantity of blood flowing through the renal vessels per minute was doubled. We noticed repeatedly that following the injection of the pituitary hormone, the venous blood was arterial in color owing to the rapidity of the flow. In some instances in which but little diuresis could be induced, the blood flow also showed but little change even though the blood pressure was raised.

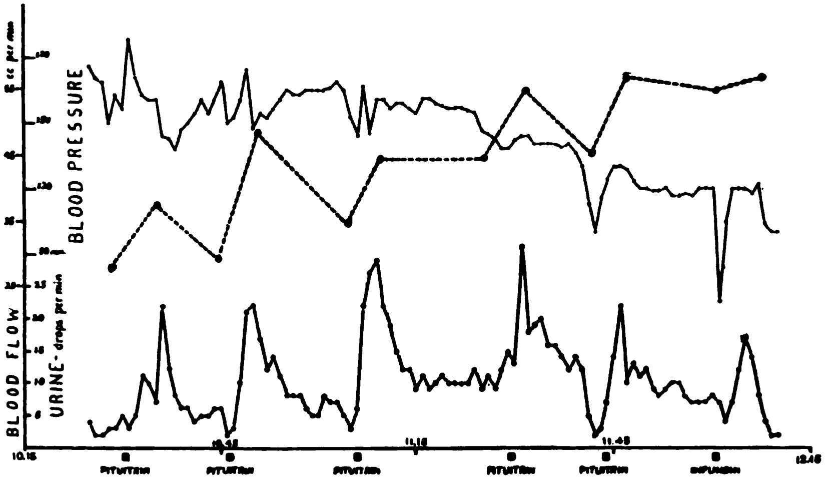


Fig. 2

Data concerning the influence of pituitrin on the oxygen consumption by the kidney are given in table 3. It may be seen that totally independent of the extent of the diuresis induced, the amount of oxygen used by the kidney remained uninfluenced by the pituitary hormone. In no successfully completed experiment was there evidence that pituitrin caused increased work on the part of the renal cells as measured by their oxygen intake.

In several instances as a check on our method we compared the effect of the pituitary extract with that produced by urea or sulphate (table 4 and fig. 3). In sharp contrast to pituitrin, the urea and sodium sul-

TABLE 2

Effect of injections of pituitary extract on blood pressure, blood flow and urine flow

EXPERIMENT	TIME	BLOOD PRESSURE MM. HG. (CAROTID)	BLOOD FLOW THROUGH KIDNEY (CUBIC CENTIMETERS PER MINUTE)	URINE (DROPS PER MINUTE)	REMARKS
1	10.40	180	17.64	Occasional drop	Animal not eviscerated
	11.16	182	36.54	11	After 0.5 cc. pituitrin
	11.35	142	23.76	7	
2	11.00	138	48.0	6	Animal not eviscerated
	11.21	124	59.4	10	Pituitrin, 1 cc.
	11.59	112	48.0	6	
3	10.34	166	36.8	9	Animal not eviscerated
	11.37	140	90.0	20	Pituitrin, 1 cc.
4	11.50	138	34.0	0	Animal eviscerated. Urine flow from one kidney
	12.21	140	66.0	10	Pituitrin, 2 cc. (two injections, 1 cc. each)
5	10.33	154	22.3	6	No evisceration
	10.50	146*	33.9	13	5 min. after 1 cc. pituitrin
	11.35	122	27.6	Occasional drop	
6	11.03	158	23.0	0	Animal eviscerated
	11.15	154	32.3	5	5 min. after pituitrin
	12.20	100	21.0	0	
	12.25	118	23.0	5	After 2nd injection, 1 cc.
7	10.47	148	19.0	0	Animal not eviscerated
	11.10	144	37.5	2	1.5 cc. pituitrin
	11.58	102	30.7	2	
	12.11	82	31.5	2	After 2nd injection, 2 cc. pituitrin
8	11.05	164	19.0	1	Animal not eviscerated
	11.17	150	20.2	4	1 cc. pituitrin
	12.00	62	11.9	Occasional drop	
	12.11	80	15.0	2	2 cc. pituitrin

* Pressure earlier had reached 170 mm.

TABLE 2—*Concluded*

EXPERIMENT	TIME	BLOOD PRESSURE MM. HG. (CAROTID)	BLOOD FLOW THROUGH KIDNEY (CUBIC CENTIMETERS PER MINUTE)	URINE (DROPS PER MINUTE)	REMARKS
9	11.05	162	15.0	2	Animal eviscerated
	11.30	146	24.0	7	1 cc. pituitrin
	12.23	72	10.7	1	
	12.40	76	10.3	2	2 cc. pituitrin
10	12.47	98	19.4	0.5	Animal eviscerated
	1.07	124	38.4	5	1 cc. pituitrin injected
11	3.42	118	24.5	5	Animal eviscerated
	3.52	120	30.0	20	1 cc. pituitrin
12	3.43	84	20.0	3	Cat with tubular nephritis. Eviscerated.
	3.59	106	23.4	7	Injected 1 cc. pituitrin

TABLE 3

Influence of pituitary extract on the oxygen consumption of the kidney

EXPERIMENT	OXYGEN CONSUMPTION (CUBIC CENTIMETER PER GRAM PER MINUTE)		URINE FLOW	REMARKS
	Before injection	After injection		
1	0.118	0.109	Doubled	Animal not eviscerated
2	0.042	0.043	Slight increase	Animal not eviscerated
3	0.061	0.047	Increased fourfold	Animal eviscerated
4	0.068	0.066	More than doubled	Animal eviscerated
5	0.068	0.041	No increase in urine	Same animal as in 4; 50 min. later
6	0.047	0.049	Increased fourfold	Animal eviscerated
7	0.045	0.035	Very slight increase	Considerable loss of blood during operation
8	0.096	0.081	Definite increase	Animal eviscerated
9	0.045	0.037	No increase	
10	0.026	0.025	Doubled	Nephritis (tubular)

phate produced the decided increase in oxygen intake previously described by Barcroft and Straub.

Two special instances encountered during our experiments also suggest that the pituitary hormone and urea produce their effects through different mechanisms. In one case although the pituitary extract produced a definite increase in urine, subsequent injection of sodium sulphate failed to cause its usual marked effect. Later inspection showed that the kidneys were pathological and microscopical examination showed a marked tubular type of nephritis. It may be noted also that the oxygen consumption by this nephritic kidney was the lowest that we have measured. In another instance repeated injections of pituitrin failed to cause any urine flow. The subsequent injection of sodium sulphate was followed by a copious diuresis. Of course one can not put too much emphasis on such evidence yet the data fit in so well with the general picture that they at least warrant mention.

TABLE 4

Influence of urea and of sodium sulphate injections on the oxygen metabolism of the kidney

EXPERIMENT	SUBSTANCE INJECTED	OXYGEN CONSUMPTION (CUBIC CENTIMETER PER GRAM PER MINUTE)		URINE FLOW	REMARKS
		Before injection	After injection		
11	Urea, 2 gm. in 10 cc. saline	0.046	0.098	Good	Following pituitrin which caused no increase in oxygen intake
12	Sodium sulphate 20 cc. of 10 per cent solution	0.013	0.045	Marked	
13	Sodium sulphate, 10 cc. of 10 per cent solution	0.031	0.090	Marked	Preceding injection of pituitrin failed to cause increase in oxygen intake
14	Sodium sulphate, 5 cc. of 10 per cent solution	0.025	0.056	Slight	Later injection of pituitrin caused on increase of O_2 intake

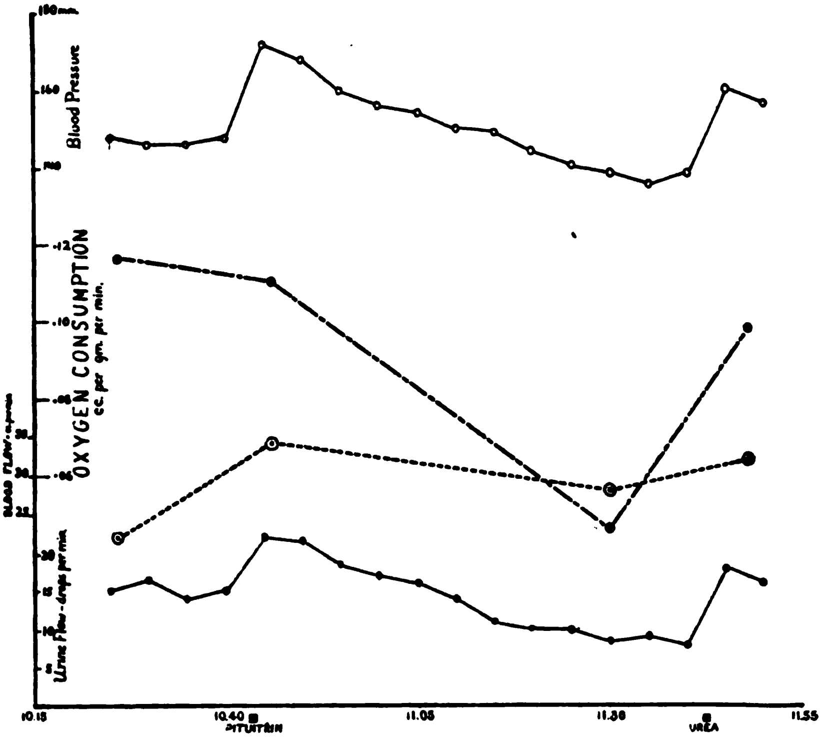


Fig. 3

DISCUSSION AND SUMMARY

That stimulation means increase of activity is a biological axiom. This increased activity always involves proportionate increase in metabolism. It is this metabolism which causes the demand for oxygen on the part of a tissue or organ. We may therefore regard the oxygen intake of an organ as a satisfactory criterion of stimulation. As Barcroft (14) has said, "There is no instance in which it can be proved that an organ increases its activity under physiological conditions without increasing its demand for oxygen." Further, "An advantage of the assurance that every increase in the activity of the cell means an instant call for oxygen lies in the fact that it furnishes a method of deciding whether in certain cases there is or is not increased activity on the part of the cell." This test for cell activity has been applied to the kidney by Barcroft and his coworkers. Thus the question of stimulation has been answered in the affirmative for such diuretic substances as urea, caffein, sodium sulphate and phloridzin, and in the negative for the diuresis induced by Ringer's solution. We accordingly applied this test to the pituitary diuresis as it seemed to us that if we obtained a definite increase in the oxygen intake it could be regarded as a positive confirmation of Schäfer's conclusion.

Our results are in accord with Schäfer's in so far as they show that at times there is complete lack of parallel between the blood pressure and the urine flow. However the argument that this furnishes conclusive proof of the stimulation of the renal cells would seem to lose its force in the light of our findings relative to oxygen consumption and blood flow. It is difficult to explain the failure of the kidney to show any change in its metabolic activity on any basis of renal stimulation. The source of the energy must lie outside the kidney. While such possibilities as physical changes in the blood or changes in the permeability of the glomerular filter can not be entirely ignored, the relations of blood flow and urine flow are such that the effect may be satisfactorily explained as due entirely to the vascular change. Dilatation of renal vessels with more or less constriction elsewhere must be the causal accompaniment of increased blood flow. This by increasing the pressure in the capillaries of the glomerulus increases the rate at which the urine is formed. That the kidney vessels do show this specific dilatation in concentrations which cause constriction elsewhere is indicated by the work previously cited (7), (8), (9). Nor is this difference in the response of the renal vessels limited to their behavior toward pituitrin. Gottlieb and Magnus (15) state that digitalis has less constrictor effect on the renal vessels than on the mesenteric vessels and Bayliss (16) found that when the depressor nerve is stimulated the change in kidney volume and blood pressure are parallel while the volume of other internal organs increases. This he interprets as indicating a different organization of vasomotor mechanisms in the kidney as compared with other internal organs.

The outstanding effects of injections of extracts of the posterior lobe of the pituitary body are on involuntary muscle. What were earlier supposed to be evidences of stimulation of gland cells (e.g., the mammary) have more recently been explained as due to stimulation of the smooth muscle of the gland (17). We believe, on the basis of our results, that the action of the kidney is of the same general nature with the difference that in the case of the renal vessel the extracts caused inhibition (i.e., vasodilation) of the smooth muscle instead of stimulation. Our results do not indicate that it is necessary to include a further effect on the cellular elements of the tubules. This vasodilation could hardly be passive as increased blood flow was found without accompanying rise of blood pressure. Several recent writers (18), (19) have reported that subcutaneous injections of pituitary extracts cause no change or may cause diminished production of urine during twenty-four hour periods. The question then of whether diuresis is produced, such as is usually obtained by intravenous injection of rather large doses, or whether there is diminished flow of urine is a question of whether the kidney vessels are dilated and an increased blood flow thereby maintained. It may possibly be found that when slower absorption occurs as from subcutaneous administration the renal dilatation may not be a prominent feature of the action; or when studied over long periods initial dilatation may be followed by a more prolonged constriction. Such results do not in any way disprove our findings.

The results of our experiments do not incline us toward the view that the posterior lobe extracts yield several hormones. The preparations that we used contained different amounts of the vasodilator substance but this is undoubtedly not specific but identical with that found in many organic extracts. The lack of parallel between the pressor effect on blood pressure and the effect on the kidney can well be explained on the basis of differing threshold of stimulation by the same active hormone.

CONCLUSIONS

1. The oxygen consumption by the kidney is not increased during the diuresis induced by pituitary extracts.
2. Using the oxygen consumption as the criterion, there is no evidence that pituitary extract stimulates the renal cells.
3. Throughout our experiments increased blood flow through the kidney was an invariable accompaniment of pituitary diuresis.
4. From the evidence at hand it seems possible to explain the diuretic action of pituitary extract entirely on the basis of the vascular changes and increased filtration pressure obtaining in the kidney.

BIBLIOGRAPHY

(1) Magnus and Schäfer: Journ. Physiol., 1901, xxvii, 9.
(2) Schäfer and Herring: Phil. Trans. Roy. Soc., 1908, 1.
(3) Schäfer: The endocrine organs, London, 1916.
(4) Hoskins and Means: Journ. Pharm. Exper. Therap., 1913, iv, 435.

(5) HOUGHTON AND MERRILL: Journ. Amer. Med. Assoc., 1908, li, 1849.
(6) KING AND STOLAND: This Journal, 1910, xxxii, 405.
(7) PAL: Wien. Med. Wochenschr., 1909, lix, 137.
(8) COW: Journ. Physiol., 1911, xlii, 134.
(9) MCCORD: Arch. Int. Med., 1911, viii, 609.
(10) BARCROFT AND BRODIE: Journ. Physiol., 1905, xxxiii, 67.
(11) BARCROFT AND STRAUB: Journ. Physiol., 1910, xli, 145.
(12) BARCROFT: Journ. Physiol., 1908, xxxvii, 12.
(13) MATHISON: Journ. Physiol., 1911, xlii, 471.
(14) BARCROFT: The respiratory function of the blood, Cambridge, 1914.
(15) GOTTLIEB AND MAGNUS: Arch. f. Exper. Path. u. Pharm., 1909, lx, 360.
(16) BAYLISS: Principles of general physiology, London, 1915.
(17) SCHÄFER: Quart. Journ. Exper. Physiol., 1915, viii, 379.
(18) MOTZFELDT: Journ. Exper. Med., 1917, xxv, 153.
(19) REES: This Journal, 1918, xlv, 471.

FURTHER STUDY ON THE EFFECT OF FOOD IN INCREASING OXIDATION

W. E. BURGE AND A. J. NEILL

From the Physiological Laboratory of the University of Illinois

Received for publication June 12, 1918

In a previous publication we (1) pointed out that the ingestion of the foodstuffs increased the catalase of the blood and hence of the tissues by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme and accordingly suggested that the increase in oxidation after the taking of food, as observed by Lavoisier, was due to the increase in catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide. It was also found that of the foodstuffs, meat was a more effective stimulant to the production of catalase than either fat or sugar and hence we suggested that the specific dynamic action of protein as set forth by Rubner (2) was due to this fact. The object of the present investigation was to determine the effect of the ingestion of the ordinary food materials, fruits and beverages on the production of catalase. The fatty foods used were bacon, lard, olive oil, glycerine and sodium palmitate; the protein foods, eggs, casein, peptone, beef extract and aminoids; the carbohydrates, starch, rice flour, corn meal, wheat flour, dextrine, honey, sucrose, maltose, lactose and dextrose; the fruits, oranges, bananas, lemons, rhubarb, apples, grape fruit; the beverages, chocolate, coffee, milk, cocoa and alcohol. After etherizing the animals and opening the abdominal wall, these substances were introduced in liquid form in about equal quantities into the stomach and intestine under pressure from a bottle through a rubber tube and an attached hypodermic needle. The quantity of the different substances used will be given in the description of the individual experiments. The catalase in 0.5 cc. of blood taken directly from the liver, the portal and jugular veins was determined before as well as at fixed intervals after the introduction of the different materials. The blood from the liver was collected from a superficial incision made in this organ. The catalase was determined by adding 0.5 cc. of blood to 50 cc. of hydrogen peroxide in a bottle at approximately 22°C. and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted burette, previously filled with water. After the volume of gas collected as described in ten minutes had been reduced to standard atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood. The material was shaken in a shaking machine at a fixed rate of one hundred and eighty double shakes per minute during the determinations. It will be noticed that none of the experiments lasted more than two hours owing to the fact that the animal with its abdominal wall open and having ether administered began as a rule to develop the condition of shock and on this account the catalase began to decrease.

In figure 1 under lard, bacon, olive oil, glycerine, sodium palmitate, trimethylene glycoll and ethyl alcohol are given curves constructed from data obtained from dogs before as well as at fixed intervals after the introduction of these materials into the alimentary tract. The lard was introduced in the form of an emulsion which was made by shaking 500 grams of lard heated to 40°C. with 200 cc. of a 1 per cent sodium carbonate solution. Five hundred grams of finely ground bacon were tried out and introduced into the alimentary tract. Four hundred cubic centimeters of glycerine were shaken with 200 cc. of water and introduced as such. The sodium palmitate was prepared by adding sodium hydroxid to 350 grams of palmitic acid. Five hundred cubic centimeters of 50 per cent trimethylene glycoll and 500 cc. of 45 per cent ethyl alcohol were used. All the substances were warmed to 38 C. previous to being introduced into the alimentary tract of the dogs. The figures along the ordinate in chart 1 as well as in all the charts in this paper represent amounts of catalase measured in cubic centimeters of oxygen liberated by 0.5 cc. of blood from 50 cc. of hydrogen peroxide in ten minutes, and the figures along the abscissa, time in minutes. The continuous line curves in all the charts of the paper were constructed from data obtained from blood taken from the liver, the discontinuous line curves from the blood of the portal vein, and the dotted line curves from the blood of the jugular. It will be seen in figure 1 that previous to the introduction of the lard, 0.5 cc. of the blood of the liver liberated 74 cc. of oxygen from hydrogen peroxide while 0.5 cc. of blood from the portal and jugular veins liberated 70 and 69 cc. of oxygen respectively. After the introduction of the lard the catalase in the blood of the liver, portal and jugular veins was increased as is indicated by the increase in the amount of oxygen liberated from

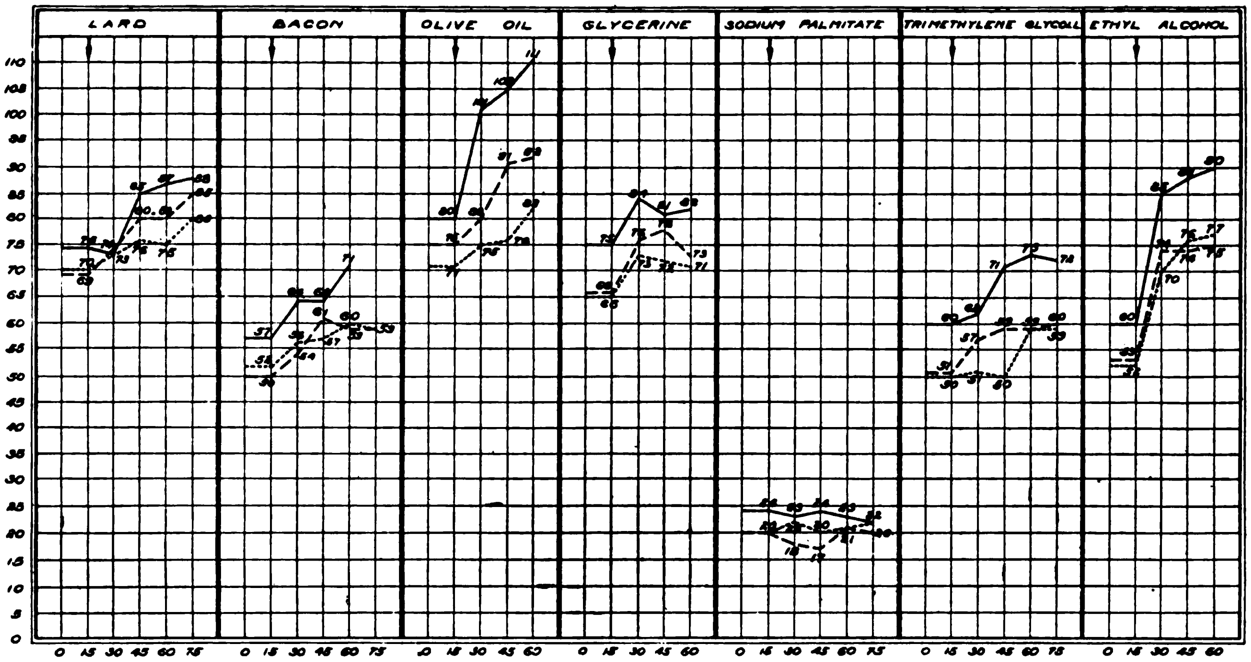


Fig. 1. Curves showing effect of the introduction of lard, bacon, olive oil, glycerine, sodium palmitate, trimethylene glycoll and ethyl alcohol into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of portal vein; - - - - - curves show amount of catalase in the blood of jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

hydrogen peroxide. The catalase of the portal blood was increased more rapidly particularly during the first thirty minutes than that of the jugular. This is taken to mean that the lard was stimulating the alimentary glands to an increased output of catalase while the increase in the catalase of the blood of the jugular was due principally to the stimulation of the liver to an increased output of this enzyme. That the liver is normally putting out catalase continuously into the blood is shown by the fact that the blood taken directly from the liver is always richer in catalase than the blood from any other part of the body by 15 to 20 per cent. The fact, as shown in a previous publication, that the introduction of a substance such as ethyl alcohol which normally produces a great increase in the catalase of the blood, produced a very small increase after the liver was cut out of the circulation by an Eck fistula and ligating the hepatic artery, was taken to mean that the liver is the organ principally responsible for the production of catalase. It may also be seen that the introduction of the bacon as well as the olive oil into the alimentary tract produced an increase in catalase. The introduction of glycerine produced an increase while sodium palmitate did not. This observation is interpreted to mean that the glycerine radical in the fat molecule is responsible for the stimulating effect of fat on the output of catalase. By comparing the stimulating effect of glycerine, trimethylene glycoll and ethyl alcohol to an increased production of catalase it will be seen that glycerine, a trihydroxy alcohol, was least effective, trimethylene glycoll, a dihydroxy alcohol was more effective, and ethyl alcohol, a monohydroxy alcohol, was most effective.

In figure 2 the curves were constructed from data obtained before as well as at fixed intervals after the introduction of eggs, casein, peptone, beef extract and biuret-free aminoids. Twelve eggs were beaten up and strained through two thicknesses of cheese cloth and introduced in this form. The casein was prepared by dissolving 400 grams of a commercial preparation of this material in 200 cc. of sodium carbonate; the peptone by dissolving 350 grams in 300 cc. of water; the beef extract by dissolving 300 grams of Liebig's beef extract in 200 cc. of water, and the aminoids by dissolving a biuret-free commercial preparation in 200 cc. of water. The aminoids contained 11.98 per cent of nitrogen and of this 8.9 per cent was amino nitrogen. It will be seen that the introduction of the egg as well as the casein produced little or no increase in the catalase of the blood as is indicated by the fact that there was no increase produced in the amount of oxygen liberated from hydrogen peroxide. The peptone and beef extract produced an increase but not so marked as did the aminoids. It is assumed that the egg and casein did not produce an increase because the length of the experiment was not sufficient to permit the digestion and absorption of these substances, while the peptone, beef extract and aminoids, being immediately absorbed, stimulated the digestive glands, particularly the liver, and thus produced an increase in catalase. According to Pawlow beef extract functions in stimulating the gastric glands to an increased output of gastric juice. The preceding observation shows that beef extract also stimulates the liver to an increased output of catalase. The fact that the aminoids being composed mostly of amino acids

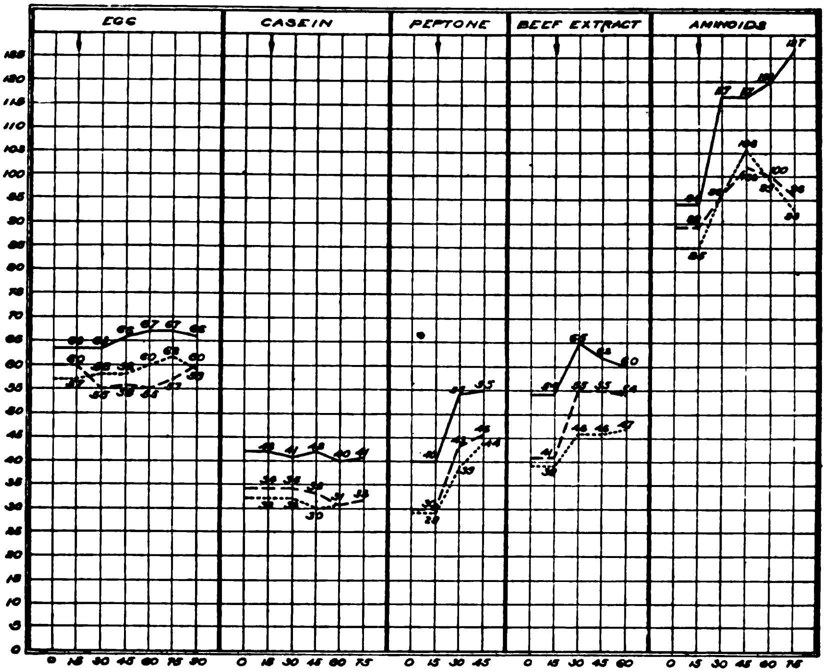


Fig. 2. Curves showing effect of the introduction of egg, casein, peptone, beef extract and aminoids into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

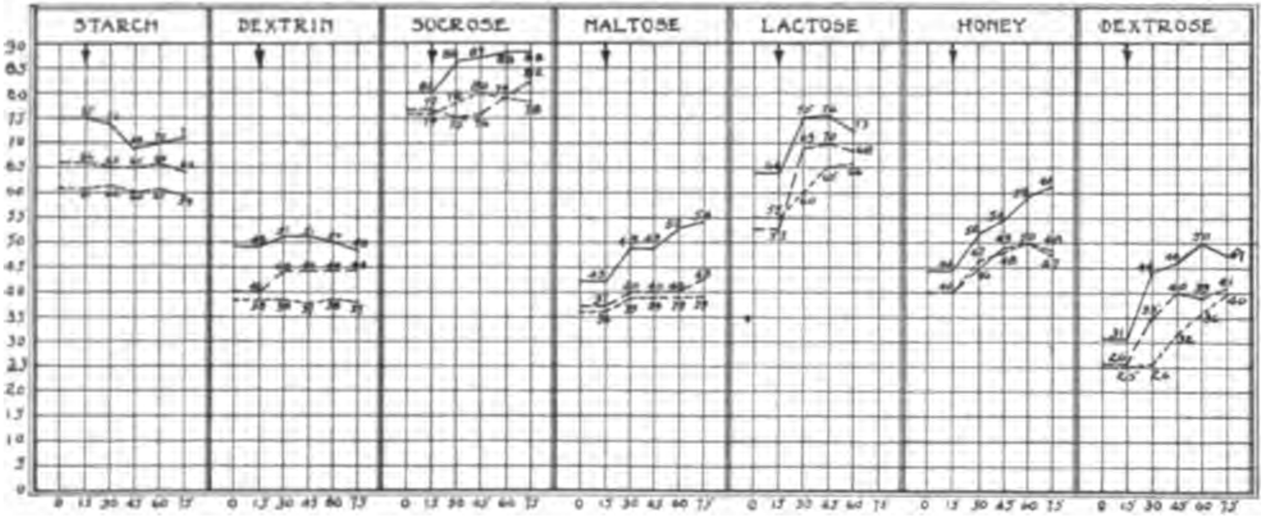


Fig. 3. Curves showing effect of the introduction of starch, dextrin, sucrose, maltose, lactose, honey and dextrose into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

stimulated the digestive glands to the greatest extent suggests that the amino acids are the substances responsible for the great stimulating effect of meat on the production of catalase.

In figure 3 under starch, dextrine, sucrose, maltose, lactose, honey and dextrose are given curves constructed from data obtained from dogs after the introduction of these materials into the alimentary tract. The starch was introduced in the form of a paste which was made by adding 200 cc. of water to 400 grams of corn starch. Four hundred grams of dextrine as well as each of the sugars used were dissolved separately in 200 cc. of water. It will be seen that the introduction of the starch as well as the dextrine produced little or no increase in the catalase of the blood while the sugars produced an increase, lactose and dextrose producing the greatest increase. It is assumed that the starch and dextrine did not produce an increase because the length of the experiment was not sufficient to permit the digestion and absorption of these substances. The dextrose produced a more rapid and extensive increase in catalase than did sucrose or maltose due presumably to the greater rapidity with which it is absorbed. Why lactose, a disaccharide should have produced a more rapid and extensive increase than the other two disaccharides, we are unable to say. However the purposefulness of this becomes evident when it is considered that lactose is the sugar on which suckling animals live. The stimulating effect of honey was due presumably to the simple sugars constituting the bulk of this substance.

In figure 4 are shown curves constructed from data obtained before as well as at fixed intervals after the introduction of rice flour, corn meal, wheat flour, dextrine and dextrose. These substances were prepared for injection by adding 200 cc. of water to 400 grams of each of the materials and warming to 38°C. It will be seen that the introduction of none of these substances except dextrose produced an increase in catalase, due presumably to the length of time of the experiment being too short to permit the digestion and absorption of the materials.

In figure 5 under oranges, oranges (very ripe), bananas, lemons, rhubarb, apples and grape fruit are given curves constructed from data obtained from dogs previous to and after the introduction of these materials into the alimentary tract. Two dozen oranges were peeled and ground to a very fine consistency. Half of this material was used immediately while the other half was permitted to stand in a thermostat at 38°C. for twenty-four hours. The material that had stood in the thermostat is referred to in the chart as oranges (very ripe). Twelve bananas were peeled, macerated and also placed in a thermostat for twenty-four hours at 38°C. The pulp and the juice of twelve lemons and of four large grape fruit were ground up separately and strained through a cheese cloth. Similarly 500 grams of rhubarb and of apples were prepared. It may be seen that the introduction of the oranges produced no increase in catalase while the introduction of oranges (very ripe) as well as the bananas which had undergone the ripening process produced an increase in the catalase of the blood. The stimulating effect of the fruit which had undergone the ripening process is attributed to the large amount of sugar and possibly alcohol in this material. The introduction of the macerated lemons and apples produced very little if any increase while the rhubarb and grape fruit produced a very small increase in catalase.

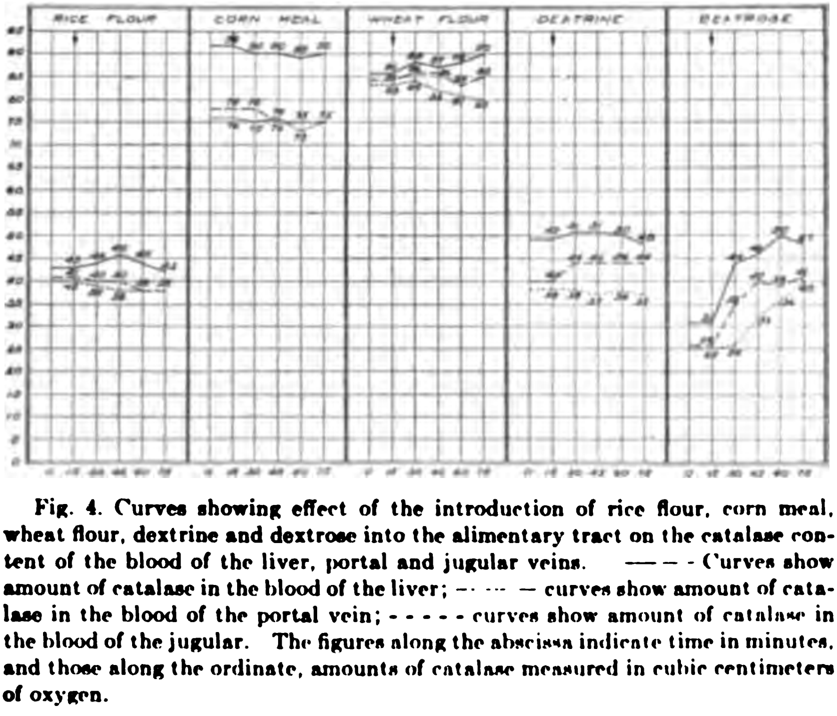


Fig. 4. Curves showing effect of the introduction of rice flour, corn meal, wheat flour, dextrine and dextrose into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— - Curves show amount of catalase in the blood of the liver; -· ··· — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

The chocolate and cocoa used in the experiments in figure 6 were prepared by dissolving 200 grams of Baker's chocolate and 200 grams of cocoa in 300 cc. of water. The coffee was prepared by adding 50 grams

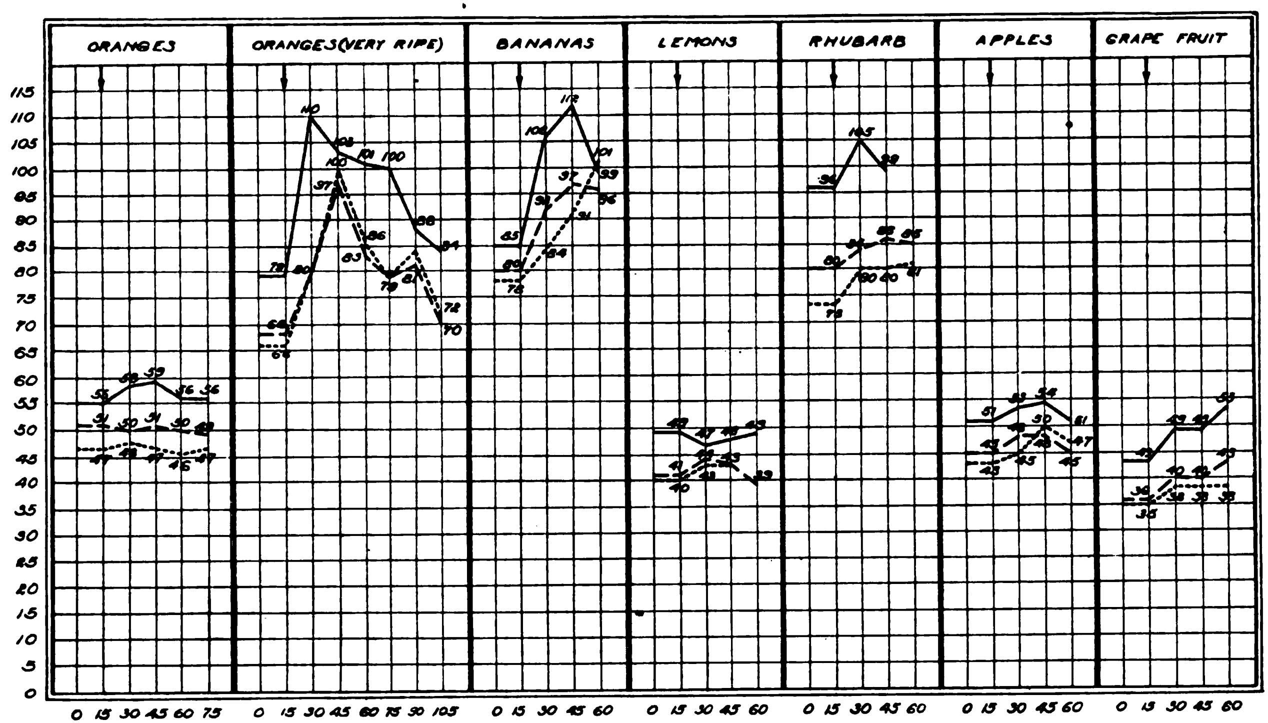


Fig. 5. Curves showing effect of the introduction of oranges, oranges (very ripe), bananas, lemons, rhubarb, apples and grape fruit into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

of this material to 400 cc. of water and boiling. The milk used was 500 cc. of fresh cow's milk. It will be seen that the chocolate produced a marked increase in the catalase of the blood, the coffee an increase in the catalase of the portal blood and the milk a very slight increase, while the cocoa produced no increase at all. The only difference between the cocoa and chocolate used was that the former contained much less fat than the latter, hence the stimulating effect of the chocolate must have been due to the presence of the fat. The increase in catalase produced by the coffee must have been due to the stimulation of the alimentary glands since the increase was confined entirely to the portal blood. Forty-five minutes after the introduction of the cocoa when it had produced no increase in the catalase, 500 cc. of 45 per cent ethyl alcohol were introduced which, as may be seen, produced a marked increase in catalase.

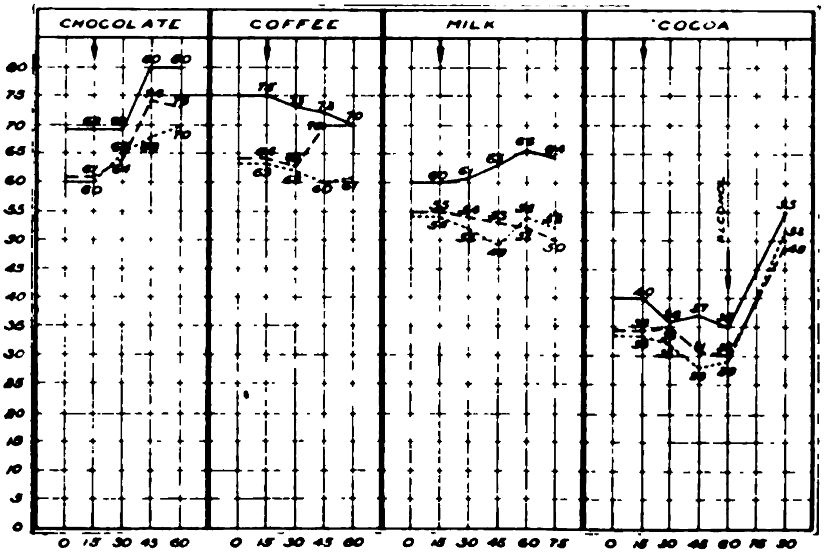


Fig. 6. Curves showing effect of the introduction of chocolate, coffee, milk, cocoa and alcohol into the alimentary tract on the catalase content of the blood of the liver, portal and jugular veins. ——— Curves show amount of catalase in the blood of the liver; — — — curves show amount of catalase in the blood of the portal vein; - - - - - curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes, and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

Lavoisier showed that the ingestion of food increased oxidation in the body. Rubner showed that of the foodstuffs meat ingestion increased oxidation most, fat next and sugar least. Magnus-Levy (3) showed that the ingestion of 320 grams of fat bacon increased metabolism 19 per cent and the ingestion of 210 grams of butter increased it 14 per cent. Johansson, Billström and Heijl (4) showed that ingestion of the simple sugars increased metabolism. Means, Aub and DuBois (5) showed that the administration of large doses of caffeine to normal individuals produced an increase in the basal metabolism 10 to 30 per cent. Lusk (6) showed that the ingestion of olive oil as well as amino acids increased metabolism and accordingly suggested that the stimulating effect of protein to increased heat production is due to the influx of amino acids or organic acids resulting from the deamination of the amino acids. Benedict (7) claims that the ingestion of carbohydrates increases oxidation by the formation of acid intermediary products which stimulate metabolism. Voit (8) believed that the presence of increased quantities of food materials augmented the inherent power of the cells to metabolize, while Rubner contended that the increased heat production which follows the taking of food was due to heat developed from intermediary reactions and oxidations which were in no way involved in the life processes of the cells. It may be seen in this paper that all of the substances which the different investigators found to increase oxidation also increased the catalase of the blood and hence of the tissues by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme. The increase in oxidation after the taking of food may be due to the increase in catalase. The fact that when oxidation is increased in the body, as for example, by increasing the amount of work, by thyroid feeding, by fighting, in the excitement stage of anaesthesia, there occurs a corresponding increase in catalase and that when oxidation is decreased, as for example, by decreasing the amount of work, by starvation, by phosphorus poisoning, in deep narcosis, in "surgical shock," or rendered defective as in pancreatic diabetes, there results a decrease in catalase, would seem to offer further evidence in this direction. It may be mentioned in this connection that we have found that when heat production was raised to higher levels by the ingestion of larger quantities of meat as observed by Rubner (9), there occurred a corresponding increase in catalase. The catalase content of the blood of a poorly fed dog was also found to be increased by feeding the animal well with the ordinary food materials. It was also found that there was an increase in the catalase of the blood during moderate exercise while there was a decrease during very severe exercise and fatigue. During the period of recovery from fatigue the catalase rose to the normal amount. If catalase is the enzyme principally responsible for oxidation in the body, it may be that the decrease in catalase with resulting decrease in oxidation is one of the factors involved in the production of fatigue and the increase a factor in the recovery from fatigue. Of the four or five hundred dogs that have been used in connection with our work on catalase during the past three years, we have observed that the catalase of the blood of lively, energetic dogs is almost invariably high while that of less energetic dogs is lower. As a rule one can pick out the dogs of high catalase on the basis of liveliness. The amount of oxygen liberated from hydrogen peroxide by the blood of sluggish dogs rarely exceeds 30 or 40 cc. and may be as low as 10 cc. in certain very sluggish dogs, while the amount liberated by the blood of lively dogs rarely falls below 70 or 80 cc. and may be as high as 175 cc. in certain very active animals.

SUMMARY

The increase in oxidation following the ingestion of food is attributed to the increase in catalase produced by the stimulation of the digestive glands, particularly the liver, to an increased output of this enzyme. The glycerine radical of the fat molecule is responsible for the stimulating effect of the fats; the end products of protein digestion, presumably the amino acids, for the stimulating effect of meat; and the simple sugars for the stimulating effect of the starchy foods.

BIBLIOGRAPHY

(1) Burge and Neill: This Journal, 1918, xlv, 500.
(2) Rubner: Energiegesetze, 322.
(3) Magnus-Levy: Arch. f. d. gesammt. Physiol., 1894, lv, 1.
(4) Johansson, Billstrom and Heijl: Skand. Arch. f. Physiol., 1904, xvi, 263.
(5) Means, Aub and DuBois: Arch. Int. Med., 1917, xix, 832.
(6) Lusk: Journ. Biol. Chem., 1912, xiii, 27, 155.
(7) Benedict: Trans. 15th Internat. Cong. of Hygiene, 1912, ii, 394.
(8) Voit: Physiologie des Stoffwechsels, 1881, 209.
(9) Rubner: Die Gesetze des Energieverbrauchs, 1902, 246.

MATERIAL LOST IN MENSTRUATION OF HEALTHY WOMEN

L. H. GILLETT, L. WHEELER AND A. B. YATES

From the Nutrition Laboratory of Teachers College, Columbia University

Received for publication June 19, 1918

In connection with metabolism experiments recently reported (1), (2), it has been possible to secure data upon the losses of nitrogen, phosphorus, calcium and iron in the menstrual flow. The results may be of interest to readers of this Journal in connection with the studies on the "periodic cardiovascular and temperature variations in women" published by King (3).

King's results show slight but irregular changes in temperature, pulse and blood pressure previous to and during the menstrual period although the changes throughout the month are often as marked as those at the menstrual period. She feels however that there is considerable consistency in the variation at this period so that her conclusion is that there is a slight but periodic movement in the physiological processes of women.

In the experiments referred to in this paper the subjects were healthy women who were working in the laboratory from ten to twelve hours a day. The main object of the experiments and the details with regard to diet have been carefully described in the papers referred to above. In general the object was to ascertain whether the calcium and phosphorus requirements of women differed materially from those of men. The diet of each subject was uniform from day to day and the daily intake and output for nitrogen, calcium and phosphorus were determined by analysis. The menstrual flow was collected on absorbent cotton, extracted frequently in tepid distilled water and the separate extractions preserved as one sample. The volume was kept as small as possible—usually below 2000 cc.—and the sample was preserved with thymol. At the end of the period samples of the solution and the used cotton were analyzed for nitrogen, calcium and phosphorus. Samples of the unused cotton were also analyzed and the amounts of nitrogen, calcium and phosphorus found in this material deducted from the amount in the used cotton. The results are shown in the following table:

TABLE I

Nitrogen, phosphorus and calcium output in the menstrual flow. These results compared with the average total daily output for the same period

	WEIGHT	LENGTH OF MENSTRUAL PERIOD	TOTAL AMOUNT IN THE MENSTRUAL FLOW			AVERAGE DAILY OUTPUT IN MENSTRUAL FLOW			AVERAGE TOTAL DAILY OUTPUT THROUGH URINE AND FECES DURING THE MENSTRUAL PERIOD		
			Nitrogen	Phosphorus	Calcium	Nitrogen	Phosphorus	Calcium	Nitrogen	Phosphorus	Calcium
	kgm.	*days*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Subject K:											
Exp. 1......	52	4	1.92	0.052	0.016	0.48	0.013	0.004	8.44	0.69	0.29
Exp. 2......	50	5	1.54	0.030	0.020	0.31	0.006	0.004	7.04	0.71	0.41
Subject M:											
Exp. 1......	57	4	3.24	0.052	0.0056	0.81	0.013	0.0014	10.86	0.74	0.22
Exp. 2.....	60	4	3.36	0.060		0.84	0.015		8.09	0.72	
Subject N:											
Exp. 1......	54	5	3.15	0.045	0.008	0.63	0.009	0.002	10.64	0.81	0.35
Exp. 2.....	55	5	1.85	0.030		0.37	0.006		6.66	0.71	

The total amount of calcium and phosphorus lost through the menstrual flow as shown by the above table is small, and while the handling of the sample is necessarily crude, the figures are at least suggestive that there is not a large loss of either of these elements at the monthly period. The nitrogen loss is larger than that of either the calcium or the phosphorus but when viewed in the light of the total output for the month is not very significant.

In experiment 2 for subject K and in experiment 1 for each of subjects M and N iron was determined on the sample with 34, 38 and 42 mgm. of iron respectively for the menstrual period of each of these three experiments. When viewed in the light of the standard for daily requirement of iron (from 10 to 15 mgms.), the loss of iron at this period is probably an important factor and should be considered by women in choosing their daily diet. The loss of iron then seemed to be more significant than any of the other elements investigated.

A study of the daily output of these six experiments (1), (2) seems to give very little basis for discussing nitrogen retention either previous to or during the menstrual period. In experiment 1, for subject K there was a noticeable retention of both nitrogen and calcium on the first day of the period. The output of nitrogen for the day preceding the period, for the four days of the period and for the day following the period is as follows: 8.84, 7.00, 9.49, 8.47, 8.80 and 8.08 grams respectively. On only two other days during the month did the nitrogen output fall below 8.00 grams, the lowest of these being 7.48 grams for the second day of the experiment. The average daily output for the month was 8.72 grams. This is the only experiment which shows a definite nitrogen retention at the beginning of the menstrual period.

In two of the six experiments the menstrual period was so near the beginning of the experiment that the output may have been influenced by the previous diet. In the three remaining experiments the nitrogen output for the first day of the period or of the preceding day was only slightly lower than for the few days previous, making a difference so small as to come within the range of experimental error and since there were several other isolated days throughout the month when the output was as low or lower than the output for this day it would be impossible to say definitely that there was a regular retention at this period. Experiments covering more than one menstrual period with several subjects would be required before definite conclusions could be drawn.

These results show as did those of King that there are as great variations during the intermenstrual periods as at the period itself and that the tendency to a variation at the menstrual period is slight

To compensate however for the diminished urinary nitrogen excretion at the beginning of the menstrual period as previously observed (4), (5), there is the extra output of nitrogen in the menstrual flow. It contains from 1.5 to 3.0 grams of nitrogen for the period, or an average of 0.3 to 0.6 gram per day during the period, which is equivalent to 0.05 to 0.1 gram per day for the month.

Of phosphorus and calcium the amounts lost through the menstrual flow were only about 0.03 to 0.06 gram and 0.01 to 0.02 gram respectively for the period—quantities which are smaller than the chance variations in the output from day to day.

The data therefore show that there is no pronounced periodicity in the output of phosphorus and calcium and that the amounts of these elements lost in menstruation are not sufficient to make the nutritive requirements of women for these elements materially different from those of men of the same weight. The figures for iron however may indicate a more significant difference.

BIBLIOGRAPHY

(1) **Sherman, Gillett and Pope**: Journ. Biol. Chem., 1918, xxxiv, 373.
(2) **Sherman, Wheeler and Yates**: Journ. Biol. Chem., 1918, xxxiv, 383.
(3) **King**: This Journal, 1914, xxxiv, 203.
(4) **Von Schroder**: Zeitschr. f. klin. Med., 1894, xxv, 72.
(5) **Mürlin**: This Journal, 1910, xxvii, 194.

MUSCULAR STRENGTH AND MUSCULAR SYMMETRY IN HUMAN BEINGS

II. In Adult Males

E. G. MARTIN and W. H. RICH

From the Laboratory of Physiology, Leland Stanford Junior University

Received for publication June 19, 1918

Determinations of muscular strength in human beings by means of dynamometers have been made in large numbers by numerous investigators in connection with various problems (1). Among the problems so approached may be mentioned studies of fatigue (2) and of relationship between physical strength and intelligence (3). The favorite instrument in these studies has been the grip dynamometer, either the old form of Collin or the improved type designed by Smedley (1). In spite of the objections that have been urged against the grip dynamometer as an instrument of research, much valuable information has been obtained with its aid (1). It presents, however, the undoubted shortcoming of making use of one of the most complex musculatures of the body and a musculature, moreover, which receives much special training. The question may properly be raised whether the strength of the grip truly represents general bodily strength or whether this particular musculature may not reflect special conditions to an exceptional degree. A test of strength, to be quite satisfactory, should afford information of the strength of the body as a whole. Ideally it should include all the important muscle-groups; practically a strength test is valid if proof can be furnished that it constitutes a fair sample of the entire strength.

The system of muscle-testing in course of presentation in the series of papers of which this is the second (4) differs from the systems previously proposed in the fundamental fact that it measures "breaking strength" of muscles, namely, the force required to overcome maximal resistance rather than the utmost positive effort, as in other methods. Which system is better can be determined finally only by continued experience and comparison. Certain advantageous features of the present system may be mentioned. As a routine feature of the test the subject is required to develop resistance at the command "hold back" uttered sharply. We find that under this stimulus the maximum effort is elicited, almost as a reflex. Only by deliberate planning on the part of the subject will less than his best effort be put forth. Thus an element of reliability is introduced, which is of the highest importance. As a matter of fact the deliberate attempt to make an inferior showing is instantly revealed in the changed character of the response. Since the spring balance with which the strength is determined is held in the

TABLE 1

Average percentage distribution of strength among the muscles, adult males. For comparison the distribution for children of 13 to 18 years is given also

MUSCLE-GROUP	ADULT MALES	13 TO 18 YEARS
Feet		
Plantar flexion	10.00	9.30
Dorsal flexion	2.85	3.20
Inversion	1.90	2.10
Eversion	1.80	2.00
Thighs		
Adduction	1.50	1.55
Abduction	1.40	1.50
Extension	3.70	3.00
Flexion	3.20	3.10
Knees		
Extension	3.30	3.30
Flexion	1.75	1.70
Shoulders		
Pectoralis	2.35	2.10
Latissimus dorsi	1.70	1.45
Anterior deltoid	2.10	2.00
Posterior deltoid	1.40	1.50
Forearms		
Extension	1.50	1.60
Flexion	2.25	2.50
Wrists		
Extension	1.05	1.35
Flexion	1.35	1.90
Fingers		
Extension	0.65	0.70
Flexion	2.95	2.75
Thumbs		
Adduction	1.30	1.40

hands of the operator, the line of pull can be rectified in accordance with the position of the subject. The maintenance of correct lines of pull is of course essential to the validity of any system of strength-testing. The promptness with which the correct position can be taken is a feature of this method which becomes valuable as a saver of time when large series of tests are being carried on.

The muscle-test. The system of muscle-testing as originally developed is described elsewhere (5). The list of muscles included in the complete test is set down in table 1. For the series of tests on adults on which this paper is based certain muscle-groups were regularly omitted. These were *a*, the plantar flexors, which are so strong in adults as to make their testing a matter of inconvenience, since a lever has to be arranged for multiplying the force of the spring-balance pull; *b*, the finger extensors, which were omitted because their short leverage requires the substitution of a special narrow sling for the full-width sling used with the other muscle-groups, a time consuming substitution, and in this particular series the saving of time was an important consideration. Tests of finger flexors were made on only part of the subjects. Here the short leverage, together with the great strength, marks the group as less satisfactory than most of the others. Tests of the wrist flexors were omitted from part of the early subjects on account of a fear, found later to be unjustified, that testing them might give discomfort.

Subjects. The adults tested were students or instructors in this University. For assistance in obtaining the coöperation of these individuals we are indebted to the Department of Military Training of Stanford University, for whose courtesy we wish to express our thanks. In addition to the fifty adult males on whom the report is primarily based, we had access to the records of an equal number of adult females tested by Doctor Mosher and one of us (6). There were also available the records of fifteen adults from the series of infantile paralysis cases reported in a former paper (4), and more than a hundred "short" tests (p. 34) made from time to time as opportunity offered. In many cases duplicate records were made at least eight hours apart; about one-fourth of the subjects were tested five or more times.

Object of the experiments. Our object in these experiments was in part the establishing for adults of the constants previously worked out for children (4). In addition to this, however, we had in mind the possibility that this system of testing might offer certain advantages for the routine determination of strength provided it could be shown to be scientifically reliable and practical in application.

The complete test, as outlined in table 1, can scarcely be carried through in less than a half-hour. Clearly, for practical purposes, some abbreviation is highly desirable. A feasible method of abbreviation would be to make actual determinations of strength of a few muscle-groups only and to compute from these the total strength. That such a method is valid is suggested in a former paper (Martin: loc. cit., p. 72). Our task here is to verify the validity of this idea and to examine the different muscle-groups individually to see which should be included in the abbreviated test. Since the first criterion of an abbreviated test

TABLE 2

Coefficients of correlation, individual muscle-groups to total strength, 56 adults

MUSCLE-GROUP	COEFFICIENT OF CORRELATION	
	Right	Left
Feet		
Plantar flexion		
Dorsal flexion	0.86	0.83
Inversion	0.68	0.66
Eversion	0.75	0.66
Thighs		
Adduction	0.815	0.81
Abduction	0.885	0.89
Extension	0.93	0.87
Flexion	0.89	0.875
Knees		
Extension	0.83	0.865
Flexion	0.87	0.93
Shoulders		
Pectoralis	0.92	0.87
Latissimus dorsi	0.73	0.76
Anterior deltoid	0.77	0.79
Posterior deltoid	0.59	0.64
Forearms		
Extension	0.825	0.82
Flexion	0.89	0.91
Wrists		
Extension	0.61	0.585
Flexion	0.60	0.64
Fingers		
Extension		
Flexion	0.47	0.50
Thumbs		
Adduction	0.80	0.78

must be its reliability, namely, the showing that it represents fairly the total strength, the muscles selected for inclusion should be those that show the highest correlation of individual strength with the total strength, provided the muscles are found to differ in this regard.

For comparing the strength of individual muscles with the total strength of the body we used a series of fifty-six cases on which complete tests (except of plantar flexors and finger extensors, as noted above) had been made. Records of wrist flexion and of finger flexion were missing also from a part of the cases. These cases were tabulated and the Pearson coefficient of correlation of each muscle with the total strength calculated. The results are set down in table 2. Inspection of this table shows that the muscles vary among themselves in the extent to which their individual strength tends to bear a fixed relation to the total strength. There are ten pairs in which the correlation of each muscle of the pair with the total strength is above 0.80, with a probable error of not to exceed ±0.032. Evidently a short test will be most reliable if selected from among these ten pairs of muscle-groups. Incidentally it may be noted that the lowest coefficients of correlation in the entire series were given by the finger flexors, which are the muscles used in tests of strength with grip dynamometers. It is probable that these coefficients are lower than would be given by tests taken with perfected instruments of the Smedley type, but the fact which seems to be generally true, to judge from table 2, that the muscle-groups with short leverage correlate less closely than do those with long leverage, indicates that the original selection of the grip as a criterion of bodily strength was perhaps unfortunate.

In making selection for a short test from among the ten pairs of muscle-groups whose correlations with total strength are satisfactorily high, the determining criterion would seem to be altogether that of suitableness for the practical procedure of the test. The application of this criterion narrows at once the range of selection. Thus hip extensors, hip flexors and knee extensors, although correlating well with total strength, are undesirable from the practical standpoint because they are very strong muscles, requiring often a tension of two hundred pounds or more to overcome their resistance, and the great labor involved in developing this high tension repeatedly is likely to prove too exhausting to the giver of the tests. The knee flexors are among the most satisfactory muscles tested on children, but with adults they are likely to cramp when contracted forcibly, a fact which constitutes a valid objection to their use in a routine test. There remain in the available list six pairs of muscle-groups, three on the legs: dorsiflexors of the foot, adductors of the thigh, abductors of the thigh; and three on the arms: pectorals, extensors of the forearm, flexors of the forearm. Minor considerations suggest the elimination from the short test of the dorsiflexors of the foot and the extensors of the forearm: the first because removal of the shoe would be necessary, the second because testing of the forearm extensors involves a degree of care in placing the parts in position which militates against speed in carrying out the test. With the idea that the test might be found available in industrial studies we have striven to reduce the time required to the least possible figure. The four pairs of muscle-groups now included in the list are the adductors and abductors of the thigh, the pectorals and the flexors of the forearm. Without any real justification but merely from a feeling that the muscles which move the hand ought to have representation in the short test, we added originally to this list the flexors of the wrist. Additional experience has convinced us, however, that the added information thus obtained is not of sufficient importance to justify the additional time consumed in testing the wrist muscles; we omit them, therefore, from our standard short test as here proposed.

For the sake of completeness, and also in order that simple directions may be readily available, the technique of the short test is here outlined.

Apparatus required. An ordinary flat-face spring-balance with a scale capacity of 200 pounds by 2 pounds, equipped with a self-registering index. (There are scales on the market with self-registering indices but these are heavier than desirable. We have found it satisfactory to fit up an ordinary scale in our own shop with a simple device.) A stout wood handle is attached by a swivel to the upper end of the scale, and a loop of stout leather 1¼ inch wide and 30 inches in circumference is attached by another swivel to the lower end of the balance.

A stout table 6½ feet long and 2½ feet wide, with a cleat secured firmly across one end. A cushion on which the subject's head may rest should be provided with this table,

An upright post 4 inches square and at least 6½ feet high, so placed that it is surrounded on at least three sides by ample space. Some form of hand-hold is provided by which the subject may steady himself as he leans against the post. (A knotted rope tied to a convenient ring near by answers well for this hand-hold.)

Procedure: General instructions. The individual to be tested is referred to as the subject. The persons giving the test are: first, the adjustor; second, the operator.

The duties of the adjustor are to place the loop in the assigned position about the arm or leg, support it there with one hand and, if necessary, the arm or leg of the subject with the other. He gives the command "hold back" to mark the beginning of the pull, and "stop" to mark the end.

The operator has the handle of the balance in his right hand and the body of the balance in his left.

After the loop is adjusted the adjustor gives the command "hold back." At this command the subject contracts with all his power the muscle-group being tested and simultaneously the operator pulls upon the spring-balance. Tension must be developed as rapidly as possible *without jerking*, and must be increased until the resistance of the subject is actually overcome. At the command "stop" the pull is discontinued immediately. The scale is read at once and the reading recorded by the assisting clerk. The sliding indicator of the scale must always be returned to the zero position immediately.

Tests are taken with the subject fully dressed.

Muscle-groups that are reported by the subject to be sore are not tested.

Calculation of total strength. The sum of the strengths shown by the individual muscles included in the short test constitutes 15 per cent of the entire strength as found by the complete test (see table 1). To calculate the entire strength, therefore, the sum of these determined strengths must be multiplied by the reciprocal of 0.15, which is 6.67. The product thus obtained is the figure for the strength of the subject. If for any reason any muscle-group was omitted from the test, assume the strength of the omitted muscle to be the same as that of the corresponding muscle on the other side.

Detailed technique of the tests. a. Pectorals. The subject stands at attention with the middle of his back pressed firmly against the upright post and the hand of the arm not being tested grasping the hand-hold. The arm to be tested is allowed to be limp in the hands of the adjustor until the command "hold back," with which command the pectoral muscles are contracted as strongly as possible. The adjustor stands directly in front of the subject, facing him; places the loop of the balance about the arm to be tested, just above the elbow; with one hand he holds the loop in position and grasps lightly the subject's hand or wrist with his other hand. Keeping the subject's arm straight, the adjustor draws it across the subject's body as far as possible, keeping it as close to the body as can be done and still give clearance for the loop. At the command "hold back" the subject's effort is to hold the arm from being drawn backward and downward from this position. The operator, standing at the subject's side, holds the balance in a line downward and backward from the subject's elbow in such a position that the arm as drawn back will just clear the subject's body. At the command "hold back" the operator develops sufficient tension to draw the arm down to the side of the body. The command "stop" must be given and the pulling discontinued before the arm has been drawn beyond the vertical line.

b. Forearm flexors. The subject lies on his back on the table with his heels pressed firmly against the cleat. The adjustor stands at the subject's left for both flexors. His right hand holds the subject's elbow to the table; his left hand brings the subject's forearm into a position of flexion about 15 degrees toward the shoulder from the vertical, and adjusts the loop about the wrist so that its upper edge is at the crease in the skin at the base of the hand. The operator stands at the foot of the table; he develops tension at the word of command. The command "stop" should be given when the forearm reaches the vertical.

c. Thigh adductors. Position of the subject same as in the above test except that he presses against the cleat only with the foot of the leg that is not to be tested. He may steady himself by grasping the edges of the table. The adjustor stands at the foot of the table; with one hand he places the loop in the hollow just above the malleolus (an equally correct index is to have the loop just clear of the top of an ordinary man's shoe); he seizes the subject's heel with the other hand; lifts the leg until the heel is just high enough to clear the other toe, and then draws the leg into extreme adduction. The toe of the leg to be tested must be kept vertical. The operator stands at the side of the table and develops tension at the word of command. The command "stop" should be given as soon as the leg is drawn into line with the axis of the body.

d. Thigh abductors. The position of the subject and of the adjustor is the same as in the above test. The loop is placed as for the adductors except that the direction of pull is opposite. The leg to be tested is drawn out 15 degrees beyond the line of the body; the effort of the subject at the command "hold back" is to prevent the operator from drawing the leg into line with the body. The command "stop" is given just as the leg reaches the midline.

The most convenient order for the tests is as follows:

Right pectoral
Left pectoral
Right forearm flexor
Left forearm flexor
Right thigh adductor
Left thigh abductor
Right thigh abductor
Left thigh adductor

Although the satisfactory giving of the test requires careful training and considerable practice on the part of operator and adjustor, the demands upon the subject are not great. We have made successful tests upon subjects with a very limited knowledge of English and only ordinary intelligence. Much time can be saved by letting subjects not yet tested see the test carried out on others. In this way they learn what is expected of them and carry out their part promptly when their turn comes. A few seconds over one minute is usually enough time for carrying out a short test.

Certain relationships which can readily be carried in mind are helpful in connection with the making of tests, especially where there is suspicion of malingering. Thus, as table 1 shows, pectorals and forearm flexors are of nearly equal strength, as are thigh adductors and abductors, and the former groups are slightly less than twice as strong as the latter. In men of ordinary strength pectoral and forearm flexors are likely to range between 75 and 100 pounds, and thigh adductors and abductors between 40 and 60 pounds. Of fifty-five adult males selected at random, 80 per cent of the muscles of the short test fell within these limits.

That the proposed short test is practicable we believe these observations demonstrate. That it gives a reliable picture of the entire strength can reasonably be assumed from the fact that each individual muscle-group included in the test correlates well with the entire strength. One would expect to find that the summed strength of these several muscle-groups would correlate with the entire strength even better than do the individual muscle-groups. That this is the case was shown by a comparison, on the same series of adults on which the correlation of individual muscles were worked out, of the summed strength of the muscles of the short test with the entire strength. The coefficient of correlation of this comparison was 0.94 ± 0.01. So high a correlation constitutes sufficient demonstration that the short test is a reliable indicator of the entire strength.

Although the expression "entire strength" as here used applies actually only to that part of the strength represented by the muscle-groups of the complete test as outlined in table 1, these observations show that in all probability the real "entire strength" bears a fixed relationship to the strength of the muscles included in the complete test and also, therefore, to the short test. The principle of "random sampling" applies here. The muscle-groups included in the complete test constitute a fairly large sample of the whole musculature. Individual muscles and small groups of muscles correlate well with the summed strength of the complete-test (see above, also Martin: loc. cit., p. 72). There is no physiological or statistical principle that would justify any other assumption than that the muscle-groups not included in the complete test correlate equally well with it and with each other.

Muscular symmetry. In the first paper of this series (4) the distribution of strength among the muscles of the body in children was shown to vary somewhat with age so that three distinct age-groups could be established (loc. cit., p. 71). The symmetry of individuals was shown to deviate from the ideals as set down in the table by varying amounts, averaging for the entire series 16.7 per cent (loc. cit., p. 79, table). Application of the method there described to the present series of adult males gives the figures for percentage strength distribution that are presented in table 1 of this paper. These figures are averages of sufficient data except for plantar flexion and finger extension; the observations on which those values were obtained are fewer than desirable. Considerable errors in these two values would affect the others of the series only slightly, however, and it is felt that they are sufficiently close approximations for present purposes.

The deviations of individuals from ideal symmetry are decidedly less in this series of adult males than in the series of children presented in the former paper. The average mean deviation for this series is 10.7 as compared with 16.7 for the former. In fact, the greatest deviation in this series, a deviation of 16.5, is less than the average for children. We were quite unprepared to find adults more symmetrical as a class than children and are inclined to think that we may have happened upon an exceptionally symmetrical group. Most of them were college undergraduates and engaged at the time when these tests were being made in intensive military training.

The relation of strength to weight. A point on which stress was laid in the former paper (4) was that the ratio of strength to weight in children tends to be constant. That the same would hold for adults seems unlikely, chiefly because so many other factors in addition to weight become operative in adults in determining strength. In the former paper (p. 74) mention was made of the fact that boys of seventeen and eighteen years show higher strength-weight ratios than do younger boys, showing the beginnings of departure from the childish condition and suggesting the incidence of additional factors.

For a study of the relation of strength to weight we had available a series of one hundred and twenty-two adult males. This included our Stanford instructors and students, a few cases from the infantile paralysis series mentioned above, and a number of recent army recruits that we were enabled to test through the courtesy and coöperation of various army officers. The group as a whole would be considered as made up of outdoor men. The Stanford students, as previously noted, were most of them taking intensive military training. The weights ranged from 107 to 196 pounds, averaging 146.6. The strengths ranged from 2000 to 5800 pounds, averaging, in round numbers, 3900. (A single individual, the college strong man, weighing 208 pounds, made a strength record of 7600 pounds. This is so far in excess of our other records that we do not include it among our averages). The ratios of strength to weight ranged from 19 to 37, averaging 26.6. We are inclined to think that our figures for average strength and average strength-weight ratio are somewhat higher than they would be for adult males in general.

As would be expected, there is a moderate correlation between strength and weight, the coefficient for this series being 0.58 ± 0.04. This accords with the familiar fact that on the whole large men are stronger than small men. The correlation is not close enough, however, to indicate that with adults, as with children, the body-weight is the dominant factor in determining strength. Whipple (loc. cit., p. 114) cites observations of correlation between strength and weight, in which strength was tested by a different method, with the following results: at Oxford, coefficient of correlation 0.46; at Cambridge, coefficient of correlation 0.56.

The significance of the strength-weight ratio. Where two individuals of equal weight differ widely in strength there are evidently at least four factors which may have influence in accounting for the difference. The first of these is actual amount of muscular tissue. There are undoubtedly considerable variations in the amounts of muscle substance present in the bodies of persons whose total weight is the same. A second factor is bodily configuration. It is quite conceivable that certain configurations lend themselves more favorably to effective exhibitions of muscular power than do others. Information on this point is lacking although it should be stated that in respect to the factor of height as an element in bodily configuration we have not been able to secure any evidence that it has significance. In investigating this point we grouped all our cases according to weight, using group intervals of five pounds, and then arranged all the members of each group in ascending order of height. We then examined the distribution of the strongest and next strongest members of each group with relation to height, and similarly of the weakest and next weakest. We found, however, nothing significant. One-half the strong were among the short men and the other half among the tall. Substantially the same distribution appeared also among the weak. Furthermore, the average strengths of the short and tall halves of each group were virtually equal.

A third factor is muscle-quality, and this undoubtedly has much to do with determining the strength. The fourth factor we may call, for lack of a better term, innervation. There can be little doubt that individuals vary in the extent to which they are able, by volition, to elicit muscular effort. That the usual manifestations fall far short of the potential maximum is shown by the familiar effects of excitement, as in the "strength of desperation." We have been at pains in the development of our procedure to avoid introducing the factor of excitement. The attempt has been to base the showing upon a maximal volitional effort made rather as a matter of routine than as a feature of competition or of desire to establish a record. Our feeling has been that a maximum effort made in "cold blood" gives a more uniform, and probably also a truer picture of the neuro-muscular power than would a similar effort made under the stimulus of excitement. On the whole, muscle quality and innervation seem to us the factors most likely to dominate in the determination of strength, and in accordance with this view we are inclined to interpret high strength-weight ratios as indicative of good muscle-quality and good innervation and low strength-weight ratos as indicative of poor muscle-quality and poor innervation. Further investigation will be necessary before these factors can be separated; indeed it is quite within the bounds of physiological possibility that they are not separable; that excellence in one feature is always bound up with excellence in the other and vice versa.

Physical classification. For convenience in assigning individuals to categories in accordance with their physical strength, some simple scheme of classification is desirable. If our assumption is correct that the strength-weight ratio is an index of muscle-quality and of innervation it would suggest itself as a sound basis on which to work out such a classification. The limits of any such classification must be more or less arbitrary, at least until sufficient data are accumulated to enable them to be established by reference to a normal probability curve. If we are correct in our assumption that the averages of our present series are somewhat higher than would hold for adult males in general, the distribution of our cases about their average is not strictly comparable with the true distribution for adult males. We feel disposed, therefore, to suggest tentatively somewhat lower limits for our proposed classes than would be indicated by our data taken by themselves. The limits of the proposed classes are given in the subjoined table.

Class	*Strength-weight ratio*
A	more than 30
B	25.1–30
C	20.1–25
D	16.1–20
E	less than 16.1

The distribution of individuals in our series of 122 is as follows: class A, 17 (14 per cent); class B, 56 (46 per cent); class C, 47 (38 per cent); class D, 2 (2 per cent); class E, none. In general terms we would say that class A includes exceptionally strong men; class B, men of more than average strength; class C, men of average strength; class D, men of less than average strength; and class E, decided weaklings. Although our series included, according to this classification, no E men and only two D men, we think it altogether likely that a more representative series, one in which clerks and factory hands were represented, would contain a due proportion of D men and a reduced percentage of A and B men. We are doubtful whether many healthy adult males will be encountered whose strength is so slight as to put them into the E class although our experience among the classes of the population in which marked physical weakness is likely to prevail is practically nil.

This classification, based on the strength-weight ratio, draws no distinction between small men of good quality and large men of equally good quality, although the latter will obviously be actually much more powerful than the former. For the practical purpose of assigning men to categories in accordance with their ability to achieve it would appear that some modification of the classification might well be made in which there is recognition of the importance of absolute muscular power as well as of good muscle-quality and good innervation. This end would be achieved if definite lower limits of strength were assigned to each class. Tentatively we would suggest the following limits: class A, 5000 pounds; class B, 4000 pounds; class C, 3000 pounds; class D, 1600 pounds. The practical effect of these absolute limits would be to require small men to show higher strength-weight ratios than large men, if they are to be placed in the higher classes. In no case would we reduce the limits suggested for the strength-weight ratios of the different classes. It would follow that a man weighing distinctly more than the average would have to show a strength well above the lower absolute limit of any given class in order to attain a strength-weight ratio that would admit him to it.

SUMMARY

1. The distribution of strength among the muscles in adult males is given (table 1).
2. The correlation between the strength of individual muscles and the entire strength is given (table 2).
3. Statistical evidence is presented showing that estimations of entire strength based on actual determinations of the strength of a few muscle-groups are valid.
4. Four pairs of muscle-groups: pectorals, forearm flexors, thigh adductors and thigh abductors are shown to correlate individually with entire strength to a satisfactory degree and to be also practically adapted for testing. They are selected, therefore, as constituting the "short" test.
5. The technique of the short test is presented in detail.

6. The summed strength of the muscles of the short test is shown to correlate well with the entire strength, the coefficient being 0.94 ± 0.01.

7. The adult males of this series are shown to have a higher average symmetry than the children of the former series; the average is 10.7 as compared with 16.7.

8. The ratio of strength to weight does not show the tendency to be constant in adult males that is seen in children. There is, however, a moderate correlation between strength and weight.

9. The factors influencing the strength-weight ratio are discussed. The conclusion is drawn that a high ratio signifies good muscle-quality and good innervation.

10. A physical classification, based primarily on the strength-weight ratio but modified to take some account of actual strength, is proposed.

BIBLIOGRAPHY

(1) For the important early references and a description of various types of dynamometers see Whipple: Manual of mental and physical tests, 2nd ed., i, Baltimore, 1914.
(2) Schuyten: Arch. d. psychol., 1903, ii.
(3) Smedley: 46th An. Rept. Chicago Bd. of Educ., 1899.
(4) Martin: This Journal, 1918, xlvi, 67.
(5) Lovett and Martin: Amer. Journ. Orthop. Surg., 1916, xiv, 415.
(6) Mosher and Martin: Journ. Amer. Med. Assoc., 1918, lxx, 140.

THE SIGNIFICANCE OF UNDISSOCIATED CARBON DIOXIDE IN RESPIRATION

R. W. SCOTT

From the Physiological Laboratory, Western Reserve Medical School, Cleveland, Ohio

Received for publication June 22, 1918

The regulation of breathing has occupied the attention of physiologists for many years but the work of Haldane and Priestley (1) in 1905 marks an epoch in the development of our knowledge of the subject. The facts they presented at that time seemed to indicate that the CO_2 of the arterial blood afforded the adequate stimulus to the respiratory center and that breathing was so regulated as to maintain at a constant level the CO_2 pressure in the alveolar air and hence in the arterial blood. It was well known, however, at the time this hypothesis was advanced that not only CO_2 but also other acids excited the respiratory center when they were added to the blood. As a result of his perfusion experiments on newborn rabbits, Winterstein (2) in 1911 formulated the hypothesis that the blood C_H was responsible for the regulation of breathing. More convincing evidence for this conclusion was given a year later by the work of Hasselbalch and Lundsgaard (3). They were able to show a definite relationship between CO_2 pressure and C_H in blood. The further work of Hasselbalch (4) on the effect of diet showed that the alveolar CO_2 pressure may be altered by several millimeters, but that the change was in such a direction as to maintain the C_H of the blood appreciably normal. These results and others led Hasselbalch to the conclusion that pulmonary ventilation is so adjusted as to maintain the C_H of the blood constant and that CO_2 excites the respiratory center only by virtue of its acting as an acid when in solution.

The work of Hasselbalch and Lundsgaard was generally accepted by the British school of physiologists led by Haldane and it has gained further support by the more recent clinical observations on the acid intoxications of diabetes and nephritis. Indeed most of the experimental and clinical data at present available seem to substantiate the hypothesis that the hydrogen ion is the true respiratory hormone. A certain security is given to this view by the nature of the relationship between CO_2 tension and C_H in blood. In the intact animal it is difficult to elicit evidence in favor of the specificity of CO_2 which might not also be attributed to the C_H. To circumvent this obstacle, highly artificial methods such as perfusion experiments have been employed by the few investigators who have attempted to show that CO_2 might act as a specific respiratory hormone. Lacqueur and Verzar (5), using Winterstein's original method of perfusing young rabbits, obtained some evidence in support of this view but their results have not been accepted. More recently Hooker (6) and his collaborators, by perfusing the isolated medulla of dogs, have found that bloods of a certain C_H with a high CO_2 tension excite the respiratory center more than blood of the same C_H containing HCl with a low tension of CO_2. The question naturally arises whether or not the results found with the very artificial preparation which these workers used would correspond to those obtained in the intact animal, in which the various tissues are concerned with the maintenance of the normal acid-base equilibrium of the body fluids. Also the criticism might be made that the C_H of the perfusate should be determined after the perfusion as well as before it. In spite of these objections the observations of Hooker are suggestive and correspond in a general way with the results reported in this paper.

At the present time there are many facts indicating that one of the important functions of respiration is to maintain at a constant level the ratio $\left(\frac{H_2CO_3}{HCO_3^-}\right)$ and hence the C_H of the blood. Any change to the acid side stimulates respiration. The tension of CO_2 during its excretion is varied, which serves as a very delicate mechanism to regulate the reaction of the blood. So far as this function of the respiratory center is concerned there is no doubt. But may the center not be affected by certain changes in the chemical composition of the blood even though there is no consequent elevation in the C_H, such for example as an increased CO_2 tension in the presence of a true alkalosis?

It is generally believed that an accumulation of free CO_2 in the blood is deleterious to the organism only in so far as it elevates the C_H, but the observations here reported show that the respiratory center resists any abnormal elevation of CO_2 tension even though the blood supplied to the medulla is distinctly more alkaline than normal. To prove this point it is of course desirable to work with the intact animal under conditions as nearly within physiological limits as possible. On the other hand, the blood must be rendered sufficiently alkaline so that when the CO_2 tension is raised the resulting change in C_H will still fall on the alkaline side of normality.

It has long been taught that the injection of alkali into an animal produces apnea. However this depends not only on the strength of the alkali but chiefly on its rate of injection. The arterial blood of an animal may be made as alkaline as P_H 7.8, yet the respiration continues in a perfectly normal fashion. In fact the tidal air per minute may be the same within a few cubic centimeters whether the P_H of the blood is 7.4 or 7.8. In such a preparation the CO_2 tension may be elevated while the C_H is appreciably more alkaline than normal. The resulting effect on the respiration cannot be attributed to any abnormal elevation in the blood C_H acting as a stimulus to the respiratory center.

Methods. Decerebrate cats were used in all the experiments reported in this paper. In order to obtain a normal response of the respiratory mechanism to a given stimulus, it is essential to avoid the depressing effects that all general anesthetics have on the center. This point was emphasized by the writer in a previous communication (7). As we have repeatedly observed, a given stimulus may evoke a definite respiratory response in the unanesthetized animal, whereas the same stimulus in an anesthetized animal produces little or no effect.

After decerebration,[1] an interval of about forty-five minutes was allowed for the elimination of the ether used before the operation. All animals that did not lie perfectly quiet or which had any irregularity in respiration were discarded. Blood samples were taken directly from either the femoral or the carotid artery with care exercised to avoid the loss of CO_2. The P_H determinations were done with the dialysis indicator method, using phenolsulphonephthalein as indicator. This method has recently been checked against the gas-chain by Clark and Lubs (8), who found that it gave results in close agreement with electrometric determinations. The results for the total CO_2 content of the blood were obtained with a modified Barcroft-Haldane apparatus, which was determined to be accurate within 1.5 per cent. The air analyses were made with the well known Haldane apparatus.

Technique of experiments. After decerebration and while the animal was respiring room air, samples of blood were taken for the P_H and total

---

[1] Details of the method of decerebration used have already been described (7).

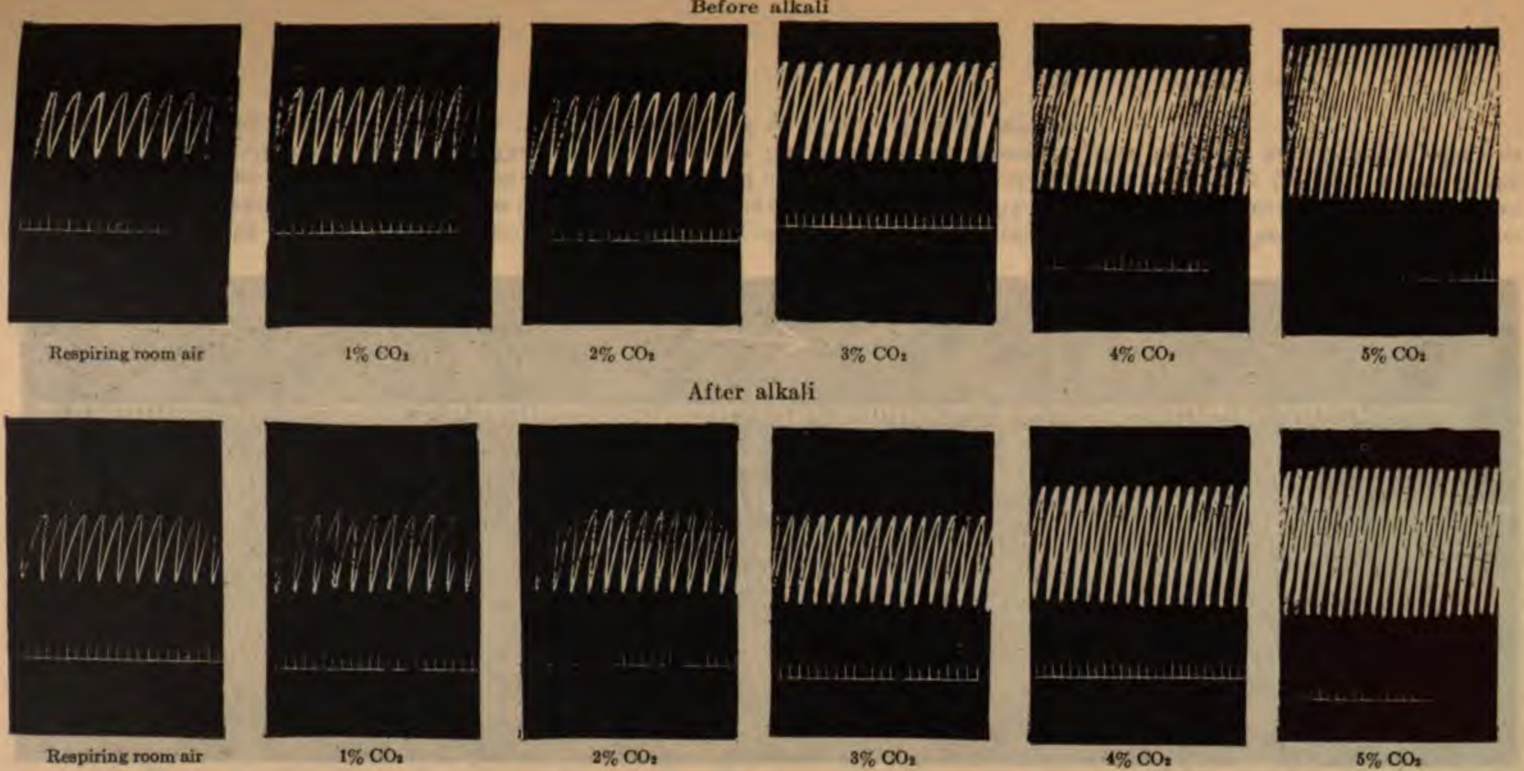


Fig. 1. Showing the respiratory records obtained in experiment 32. Upper row of tracings represents the tidal air in the normal animal corresponding to increasing percentages of inspired CO_2. The lower row was taken under like conditions from the same animal after the injection of alkali (0.35 gm. Na_2CO_3 per kg.) Note the similarity of the response in the two cases. Upstroke = expiration. Each millimeter = 4 cc. of air. Time in seconds.

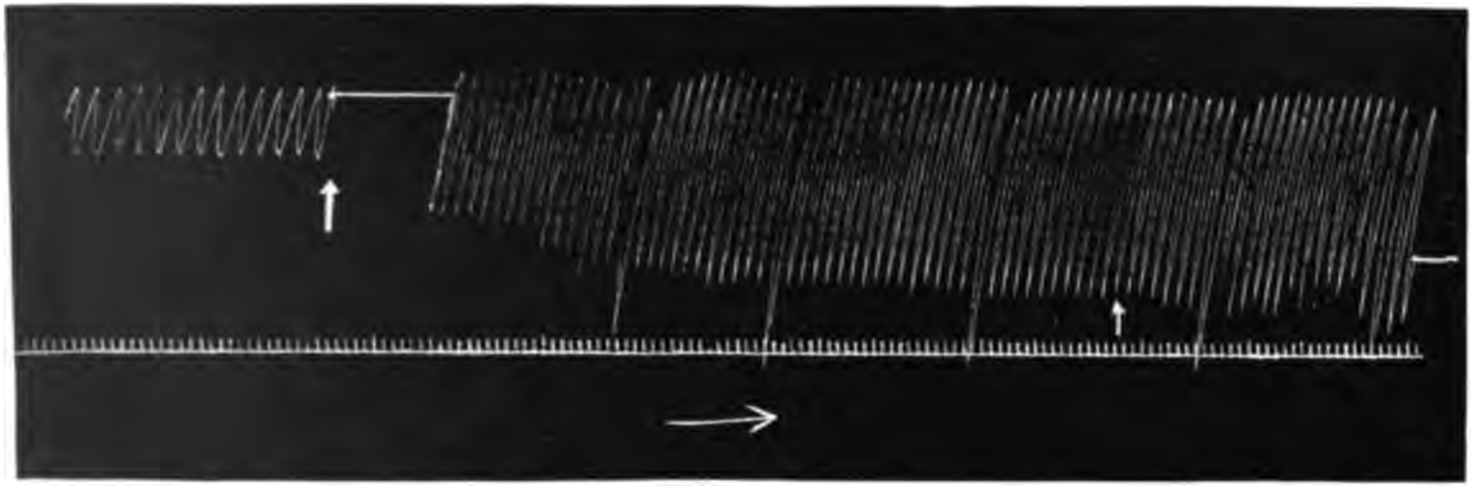


Fig. 2. Showing the effect in the alkalinized animal of inspiring air containing 7.2 per cent CO_2, (exp. 37). The first part of the tracing represents the tidal air while room air was being respired. At the pause in the tracing CO_2 was introduced. Note the stimulating effect on respiration. After inspiring the CO_2 rich air for about one and one-half minutes and at the point marked by the small arrow to the right, a sample of carotid blood was taken. The P_H was found to be 7.55, whereas the normal for this particular animal before alkalosis was 7.4. Upstroke in tracing = expiration. Each millimeter = 4 cc. of air. Time in seconds.

TABLE 1

The reaction to CO_2 *in the normal and alkalinized animal*

EXP. NO.	INSPIRED AIR	BEFORE ALKALI				AFTER ALKALI			
		Respiratory rate per minute	Tidal air per minute	P_H of arterial blood	Total CO_2 arterial blood per 100 cc.	Respiratory rate per minute	Tidal air per minute	P_H of artorial blood	Total CO_2 arterial blood per 100 cc.
			cc.		*cc.*		*cc.*		*cc.*
32	Room air..........	28	1,008	7.4	38	31	1,054	7.8	76
	1 per cent CO_2.....	30	1,260			31	1,209		
	2 per cent CO_2.....	30	1,380			32	1,344		
	3 per cent CO_2.....	35	1,960			35	1,610		
	4 per cent CO_2.....	43	3,182			38	2,356		
	5 per cent CO_2.....	50	4,400	7.2	56	46	3,496	7.5	87
33	Room air..........	29	928	7.35	36	29	940	7.75	70
	1 per cent CO_2.....	32	1,408			30	1,200		
	2 per cent CO_2.....	34	2,040			33	1,430		
	3 per cent CO_2.....	39	2,656			35	1,780		
	4 per cent CO_2.....	43	3,612			38	2,420		
	5 per cent CO_2.....	45	4,500	7.25	60	42	3,360	7.60	92
34	Room air..........	26	728	7.4	40	27	735	7.8	72
	1 per cent CO_2.....	27	820			27	800		
	2 per cent CO_2.....	29	1,000			28	870		
	3 per cent CO_2.....	30	1,640			30	1,225		
	4 per cent CO_2.....	34	2,230			32	1,640		
	5 per cent CO_2.....	37	3,256	7.25	59	34	2,183	7.55	90
35	Room air..........	24	1,056	7.4	35	26	1,000	7.70	73
	1 per cent CO_2.....	25	1,142			26	1,092		
	2 per cent CO_2.....	27	1,543			27	1,154		
	3 per cent CO_2.....	31	2,345			29	1,462		
	4 per cent CO_2.....	34	3,280			33	2,010		
	5 per cent CO_2.....	37	4,562	7.20	54	35	2,860	7.55	85
36	Room air..........	26	708	7.35		25	722	7.75	
	1 per cent CO_2.....	27	822			25	761		
	2 per cent CO_2.....	29	940			27	830		
	3 per cent CO_2.....	32	1,320			29	931		
	4 per cent CO_2.....	36	2,180			32	1,540		
	5 per cent CO_2.....	44	3,120	7.25		36	2,132	7.6	81

the reaction to CO_2 before and after alkali. The respiratory rate and and tidal air per minute corresponding to various percentages of inspired CO_2 were taken from tracings similar to the ones shown in figures 1 and 2. In table 2 are given the values for the free and combined CO_2 in the blood in the normal and alkalinized animal, first while breathing room air and again while inspiring 5 per cent CO_2. Although not representing absolute values,[2] these data are valuable in showing the relative changes which occur in the $\frac{H_2CO_3}{NaHCO_3}$ ratio of the blood under the experimental conditions. The results of the second series of more acute experiments are given in table 3. Here the respiratory data were obtained from tracings similar to the ones shown in figure 2.

TABLE 2

The relation between the free and the combined CO_2 in the blood before and after alkali

EXP. NO.	RESPIRED AIR	BEFORE ALKALI				AFTER ALKALI			
		P_H	$(H_2CO_3)+(NaHCO_3)$	(H_2CO_3)	$(NaHCO_3)$	P_H	$(H_2CO_3)+(NaHCO_3)$	(H_2CO_3)	$(NaHCO_3)$
			cc. per 100 cc.	*cc. per 100 cc.*	*cc. per 100 cc.*		*cc. per 100 cc.*	*cc. per 100 cc.*	*cc. per 100 cc.*
32	Room air	7.4	38	2.0	36.0	7.8	76	1.6	74.4
	5 per cent CO_2	7.2	56	4.5	51.5	7.5	87	3.6	83.4
33	Room air	7.35	36	2.1	33.9	7.75	70	1.7	68.3
	5 per cent CO_2	7.25	60	4.3	55.7	7.6	92	3.1	88.9
34	Room air	7.4	40	2.1	37.9	7.8	72	1.5	70.5
	5 per cent CO_2	7.25	59	4.2	54.8	7.55	90	3.3	86.9
35	Room air	7.4	35	1.8	33.2	7.75	73	1.7	71.3
	5 per cent CO_2	7.2	54	4.3	49.7	7.55	85	3.2	81.8
36	Room air	7.35				7.75			
	5 per cent CO_2	7.25				7.6	81	2.7	78.3

---

[2] These results were calculated from the equation $\frac{H_2CO_3}{NaHCO_3} = \frac{\lambda C_H}{K}$, in which the known values of the constants were those given by Michaelis and Rona (1912), $K = 4.4 \times 10^{-7}$, λ for blood conditions $= 0.605$.

Discussion of results. The data obtained before the injection of Na_2CO_3 are similar to those previously reported (7) and are presented here for comparison to show in what way a condition of alkalosis affects the reaction to CO_2. It is noted that when a 5 per cent CO_2 air is inspired, the amount of CO_2 given off by the blood is markedly elevated above normal[3] while the C_H is detectably raised above the value found when room air was being respired. Under these experimental conditions with a partial pressure of CO_2 not above 50 mm., it is probable that the increased amount of CO_2 found in the blood was for the most part bound as $NaHCO_3$, whereas a relatively small amount was carried by the hemoglobin and other blood proteins. Buckmaster (9), for example, has found that hemoglobin is capable of combining with more CO_2 than the other proteins found in blood. His figures show that at pressures between 760 and 70 mm., hemoglobin can bind considerable quantities of CO_2. But there is no conclusive evidence to indicate that this is true at the pressure of CO_2 which may obtain during life.

TABLE 3

The effect of inspiring a CO_2-rich air for one and one-half minute on respiration and the P_H of the arterial blood in the alkalinized animal

EXP. NO.	INSPIRED AIR	RESPIRATORY RATE PER MINUTE	TIDAL AIR PER MINUTE	PERCENTILE INCREASE IN TIDAL AIR PER MINUTE	P_H OF ARTERIAL BLOOD	P_H OF ARTERIAL BLOOD BEFORE ALKALI
37	Room air	32	1,152		7.8	7.4
	CO_2 = 7.2 per cent	44	4,928	327	7.55	
38	Room air	24	1,056		7.75	7.35
	CO_2 = 7.5 per cent	46	5,336	465	7.50	
39	Room air	28	840		7.75	7.4
	CO_2 = 6.4 per cent	40	4,182	400	7.60	
40	Room air	30	983		7.70	7.4
	CO_2 = 6.2 per cent	43	4,380	350	7.55	

---

[3] Recently Henderson and Haggard (Journ. Biol. Chem., 1917, xxxiii, 343) have confirmed our earlier observations by showing that the total CO_2 content of the blood is raised in a condition of CO_2 acidosis, brought about either by breathing a CO_2-rich air or by depressing the respiratory mechanism with the administration of morphine.

A very significant fact is noted in the behavior of the alkalinized animal while breathing room air. Neither the respiratory rate nor the tidal air per minute is affected to any appreciable extent by the carbonate injection, although the P_H of the blood has been changed on the average from 7.4 to 7.8. This fact indicates that a given change in P_H on the alkaline side is attended with fewer apparent disturbances than a similar change to the acid side of normality. *However, the ability of the organism to tolerate a given elevation in C_H probably depends on the acid responsible for the elevation.* Certain observations that we have made on CO_2 acidosis have indicated that the physiological limits of C_H toward the acid side are greater for H_2CO_3 than for other acids. For example, the P_H of the arterial blood in a decerebrate cat may be changed from a normal 7.4 to 7.1 by breathing air rich in CO_2. After breathing room air for twenty minutes the blood P_H and respirations have returned to normal. This acute experiment may be done as often as three times in two hours without any apparent ill effects. On the other hand, it has been found extremely difficult to reduce the P_H to 7.1 by the slow injection of acids such as N/20 HCl or N/20 acetic. In the majority of cases the animals have developed convulsive movements and died before that level was reached.

One important factor concerned in the explanation of these results is the behavior of the body bicarbonate in the two cases. We have shown that the bicarbonate content of the blood is much increased in a condition of CO_2 acidosis. For example, the total CO_2 in the arterial blood was found to increase from a normal 38.4 vols. to 77.2 in a decerebrate cat whose blood P_H was lowered from 7.4 to 7.1 by breathing air rich in CO_2 (7). On the other hand Van Slyke (10) found that after the injection of 75 cc. N/1 (NH_2SO_4) into a dog, the P_H of the arterial blood had changed from a normal 7.33 to 7.17, while the total CO_2 was diminished from 38.7 to 10.1, a reduction of over 200 per cent. These facts have much significance. They show that in the two general types of acidosis, there is a wide variation in the amount of available alkali in the blood. In CO_2 acidosis the increased quantity of $NaHCO_3$ present in the blood serves as available alkali to neutralize any further acidity. In the case of other acids the situation is different. Here the alkali is consumed and instead of actually increasing in amount it undergoes a progressive diminution in proportion to the amount of acid present. In so far then as the available alkali of the blood is used up, the ability to withstand any further acidity must be diminished. Doubtless similar circumstances prevail in the tissues, where it becomes increasingly difficult to maintain the environment within physiological limits of P_H as the bicarbonate content of the body fluids becomes depleted. These considerations alone, apart from the easy elimination of CO_2 by the lungs, indicate that the organism is better equipped to resist an increase in C_H due to H_2CO_3 than it is one caused by other acids.

A CONDITION OF TRUE ALKALOSIS NOT ACCOMPANIED BY APNEA

The absence of any respiratory disturbance accompanying a condition of true alkalosis indicates that there is not the close parallelism existing between the activity of the respiratory center and the C_H of the blood that has generally been supposed. Otherwise the degree of alkalinity present should give rise to apnea. Since the respiration continues in a normal way, it must be concluded that other factors besides the C_H may be concerned in the chemical regulation of breathing, and in this connection the free CO_2 of the blood deserves consideration.

Reference to table 2 shows that a definite increase in the blood alkalinity with only a slight change in the free CO_2 may be produced by the slow injection of Na_2CO_3. Under these circumstances the animal breathes normally. On the other hand, as is well known, apnea results when any appreciable alkalosis is caused by the methods ordinarily used, i.e., the rapid injection of alkali or superventilation of the lungs. Both procedures cause a sudden lowering in the CO_2 tension of the blood. It is therefore apparent that two types of alkalosis may be distinguished: *a*, having a normal CO_2 tension with no disturbance in breathing, as illustrated by the experiments here reported; *b*, having a lowered CO_2 tension accompanied by apnea.

The significance of a lowered CO_2 tension as the cause of apnea has been recognized for many years. Bieletzky (11) in 1882 showed that apnea was produced in birds by over-ventilation of the lungs. Later Fredericq (12), using his crossed cephalic circulation method, and Hougardy (13), by the injection of alkali, concluded that apnea was due to a diminution in the CO_2 tension of the arterial blood. A similar conclusion was reached by Campbell, Douglas, Haldane and Hobson (14) as a result of their experiments on man. They, however, interpreted their findings to accord with the hypothesis of Winterstein and Hasselbalch, attributing a preponderating influence to the C_H as the respiratory hormone. By so doing they assume that CO_2 affects the respiratory center only by virtue of its acid nature when in solution.

Accordingly a condition of alkalosis should be accompanied by apnea, which should persist until the metabolic CO_2 accumulated to such a degree as to raise the C_H to the threshold-exciting value for the respiratory center. The evidence here presented shows that this is not the case. Respiration continues regularly in the presence of a demonstrable alkalosis, provided the CO_2 tension in the blood is maintained about normal. On the other hand, we have found that apnea is very readily produced in the alkalinized animal by lowering the CO_2 tension with artificial ventilation. It is therefore clear that the apneic pause is not caused by a diminution in the blood C_H, as would be the case if the chemical regulation of breathing depended solely on blood reaction. Our results indicate a close association between apnea and CO_2 tension, thus corroborating the observations of earlier investigators. A certain significance is hereby given to CO_2, which will obviously be neglected so long as its effect on the respiratory center is considered to be an indirect one through the blood reaction.

It has been noted that the very slow injection of Na_2CO_3 has no effect on the respiration. A similar observation was made by Hougardy (13) but there was no method available at that time to determine the C_H of the blood. Reference to table 2 shows that the injection of 0.35 grams Na_2CO_3 per kilogram produces on the average a change in P_H from a normal 7.4 to 7.7 or 7.8, whereas the free CO_2 in the blood undergoes only a slight diminution. In view of the fact that Na_2CO_3 is capable of fixing CO_2 to form $NaHCO_3$, the question naturally arises concerning the mechanism by which the organism is able to maintain an approximately normal CO_2 tension in the arterial blood. Doubtless an important factor in this connection is the retention of a considerable quantity of metabolic CO_2. It is apparent that such a mechanism would afford the most available means of combating an impending alkalosis. In experiment 32, table 2, the normal total CO_2 was 38 vol. per cent; after the alkali injection it was 76 vol. per cent. Only about one-third of this increase found can be derived from the CO_2 contained in the quantity of Na_2CO_3 injected. For example 0.35 gram Na_2CO_3 contains 0.145 gram CO_2. Estimating the body fluids at 700 cc. per kilogram body weight, the total CO_2 found after the carbonate injection is equivalent to 0.476 gram CO_2 at 20°—760 mm. This value is based on the assumption that the slow rate of injection permitted a uniform distribution to the body fluids. Although we have no data on the total respiratory exchange, in all probability the disparity found is due to a retention of CO_2 that might otherwise be eliminated by the lungs. When an alkali capable of combining with CO_2 is rapidly injected, the most ready means of neutralization is by the free CO_2 in the blood. This naturally undergoes a sudden diminution, which no doubt explains the apneic pause under these circumstances. On the other hand, the slow injection of alkali allows sufficient time for the retention of metabolic CO_2 so that the free CO_2 of the blood suffers no marked diminution and consequently the breathing is unaffected.

THE EFFECT OF ALKALOSIS ON THE REACTION TO CO_2

Reference to table 1 and figure 1 shows that the general character of the respiratory response to CO_2 is the same after the injection of alkali as before. A slight difference, however, is noted in the quantity of tidal air which corresponds to a given percentage of inspired CO_2. This disparity is shown graphically in figure 3. Here the curves[4] were plotted from the data in table 1, and therefore represent the mean in five animals before and after receiving alkali. It is noted that similar percentages of CO_2 elicit a slightly more vigorous reaction on the part of the respiratory center before alkalosis is established. Furthermore, this disparity becomes more conspicuous with increasing quantities of CO_2 in the inspired air. The following facts merit consideration in explaining these phenomena: *a*, the levels at which the C_H changes; and *b*, the difference in the buffer value of the blood in normal and alkalinized animals. Reference to table 3 indicates that when the normal animal is made to breathe increasing percentages of CO_2 up to 5 per cent, both the CO_2 tension and the C_H of the blood are elevated. In this case the respiratory center may be influenced by two factors, CO_2 and C_H. After alkalosis is established only the CO_2 tension is raised by breathing 5 per cent CO_2, while the C_H always remains on the alkaline side of the normal. It is not surprising under these conditions that CO_2 might elicit a less vigorous respiratory response. In other words, the threshold value of CO_2 may be raised in a condition of alkalosis and lowered in the presence of an elevation in the C_H of the blood.

It is observed that the total CO_2 content of the arterial blood is raised by approximately 100 per cent after the injection of the quantity of Na_2CO_3 used. Consequently the buffer value must be greatly increased. The addition of a given quantity of CO_2 to such a blood will cause less elevation in the H_2CO_3 content than would be the case with normal blood. This is shown by the data in table 2. While the alkalinized animal is breathing room air, the free CO_2 is a little under the value found before the alkali injection. A similar disparity is noted when air containing 5 per cent CO_2 is inspired. From these considerations it may be concluded that both the increased buffer value and the diminution in C_H of the blood are concerned in the explanation of the fact that increasing percentages of CO_2 in the inspired air cause a somewhat less vigorous respiratory response in the alkalinized than in the normal animal. Otherwise the similarity of the response is very striking in the two cases (see fig. 3).

---

[4] The data from single experiments give curves of the same general contour.

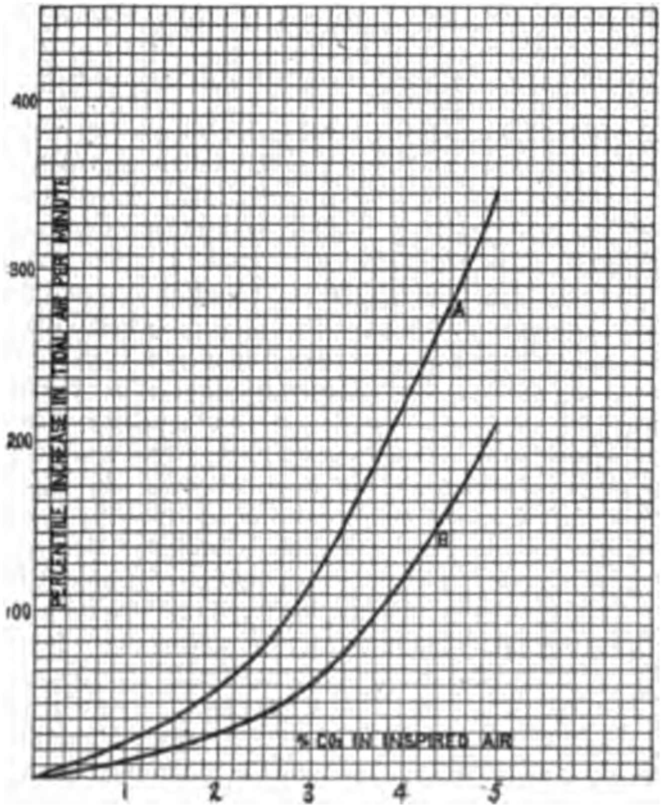


Fig. 3. Composite curves from the data obtained in five animals (see table 1) showing the respiratory reaction to increasing percentages of CO_2 in the inspired air. *A*, the normal reaction before alkali injection. *B*, after the intravenous injection of Na_2CO_3 (0.35 gram per kg.).

Table 3 shows the results obtained in the series of acute experiments in which the alkalinized animal was made to breathe a CO_2-rich air instead of accumulating the CO_2 by rebreathing. The respiratory data were obtained from tracings similar to the one shown in figure 2. When the inspired CO_2 was suddenly changed from 0.04 per cent (room air) to 6 or 7 per cent, there was a marked stimulation of the respiratory center, evidenced by an increase in the rate as well as the depth of breathing. Under these circumstances the tidal air per minute was elevated in the different animals, ranging from 327 to 465 per cent. It is noted that this marked augmentation in respiration occurred in the presence of a blood P_H that was demonstrably on the alkaline side of the normal found before alkalosis was established. Such results afford a striking illustration of the stimulating effect of CO_2 on respiration, which obviously cannot be attributed to any abnormal elevation in the C_H of the blood.

The current hypothesis recognizing the C_H as the sole factor concerned in the chemical regulation of breathing has come to be so universally accepted that little attention is paid to the effect of undissociated CO_2 on respiration. It is taught that CO_2 can influence the respiratory center only by virtue of its acid properties when in solution. Consequently an abnormal elevation in CO_2 tension in the blood is thought to stimulate respiration only in so far as the C_H is thereby raised. To quote from a recent contribution of Haldane (15),

> . . . . there seems now to be no doubt that it is true that what the respiratory center responds to is hydrogen ion concentration and not mere CO_2 pressure.

In the light of present facts there is no doubt that the C_H is one important factor influencing breathing. For example, it is well established that under normal conditions pulmonary ventilation is so regulated as to keep the C_H appreciably constant while any change to the acid side is accompanied by a marked increase in respiration. It has therefore been concluded that a close parallelism existed between pulmonary ventilation and the blood C_H. However, when we compare the activity of the respiratory center at slightly alkaline levels of C_H we find that no such definite relationship exists. Here instead of apnea resulting, as might be expected, the breathing continues regularly and is apparently directed toward maintaining the CO_2 at a normal level. Any elevation in this level causes a marked stimulation of respiration.

In view of these facts it seems probable that the so-called respiratory hormone is not a single entity in the form of the hydrogen ion as postulated by the theory of Winterstein and Hasselbalch. To assume that the respiratory center is oblivious to changes in the chemical equilibria in the blood except in so far as such changes may alter the C_H, is placing a limitation on its function which is hardly commensurate with the importance of respiration in the living animal. Apparently the center is very sensitive to undissociated CO_2, and certainly in the alkalinised animal our results indicate that CO_2 may act as a specific respiratory hormone. This gives an added importance to the physiological significance of CO_2 and at the same time illustrates a function of the respiratory center which will not be appreciated if we restrict its activity solely to the influence of the C_H.

SUMMARY

The reaction to CO_2 has been compared in the normal and alkalinized animal, using the quantitative response of respiration, together with the P_H and total carbonate content of the arterial blood in the two cases. By the slow injection of Na_2CO_3 (0.35 gram per kilogram), a demonstrable alkalosis is produced, yet the breathing continues normal indicating that a change in the C_H to the alkaline side is better tolerated than a similar change toward acidity. Examination of the arterial blood shows that by the alkali injection the P_H has been altered from the normal 7.4 to 7.8, while the total CO_2 content is elevated by approximately 100 per cent. Evidence is presented to show that the regular type of breathing seen in the alkalinized animal is intimately associated with the maintenance of the free CO_2 in the blood at a normal level, while any appreciable elevation in this level causes a marked stimulation of respiration in spite of the fact that the C_H of the blood is sensibly on the alkaline side of normality. Such observations have led to the following conclusions:

1. There is not the close parallelism between respiration and the C_H of the arterial blood supposed by the theory of Winterstein and Hasselbalch.
2. At slightly alkaline levels of C_H, pulmonary ventilation is so regulated as to maintain the CO_2 tension in the blood normal.
3. A condition of true alkalosis is accompanied by apnea only when the method used to lower the C_H causes at the same time a sudden fall in the CO_2 tension; as, for example, the rapid injection of an alkali capable of binding CO_2, or by superventilation of the lungs.

4. Undissociated CO_2 acts as a specific respiratory hormone; therefore the physiological effects of CO_2 on respiration can not be attributed solely to its acid properties when in solution.

BIBLIOGRAPHY

(1) **Haldane and Priestley**: Journ. Physiol., 1905, xxxii, 225.
(2) **Winterstein**: Pflüger's Arch., 1911, cxxxviii, 167.
(3) **Hasselbalch and Lundsgaard**: Biochem. Zeitschr., 1912, xxxviii, 77.
(4) **Hasselbalch**: Biochem. Zeitschr., 1912, xlvi, 428.
(5) **Lacqueur and Verzár**: Pflüger's Arch., 1912, cxliii, 395.
(6) **Hooker, Wilson and Connett**: This Journal, 1917, xliii, 351.
(7) **Scott**: This Journal, 1917, xliv, 196.
(8) **Clark and Lubs**: Journ. Bact., 1917, ii, 191.
(9) **Buckmaster**: Journ. Physiol., 1917, li, 164.
(10) **Van Slyke and Cullen**: Journ. Biol. Chem., 1917, xxx, 337.
(11) **Bieletzky**: Biol. Centralbl., 1882, i, 743.
(12) **Fredericq**: Trav. labor., Liége, 1889, iii, 1; Ibid., 1901, vi, 99.
(13) **Hougardy**: Arch. internat. de Physiol., 1904, 1.
(14) **Campbell, Douglas, Haldane and Hobson**: Journ. Physiol., 1913, xlvi, 301.
(15) **Haldane**: Organism and environment, New Haven, 1917.

XIV. DIFFERENCES IN THE CATALASE CONTENT OF MUSCLE FROM DIFFERENT PARTS OF THE STOMACH

WALTER C. ALVAREZ AND ESTHER STARKWEATHER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication July 9, 1918

Catalase is the ferment which liberates oxygen from hydrogen peroxid. As we have shown in previous papers, it seems to have something to do with the oxidative processes. Our work on the muscle of the small intestine suggested strongly that the catalase content of a tissue can be used as an index of its metabolic activity (1). We found that the gradient of catalase content from duodenum to ileum closely paralleled the gradients of rhythmicity, irritability, latent period and CO_2 production.

Alvarez has shown that the peristaltic waves in the stomach probably follow gradients of irritability, rhythmicity and latent period from cardia to pylorus (2). We wish to show in this paper that the catalase content of the muscle is also graded from cardia to pylorus.

TECHNIC

This has been described carefully in a previous paper (1). The animals were killed either by a blow on the head or by chloroform and bleeding. By using a shaking machine five tests on muscle from the same stomach were run at the same time under identical conditions. In the following tables the figures represent cubic centimeters of oxygen at atmospheric pressure, liberated from 15 cc. of hydrogen peroxid by the catalase in 0.3 gram of minced muscle. The muscle was peeled off of the mucous membrane. No difficulty was met with in doing this except along the lesser curvature of the rabbit's stomach. There the mucosa had to be scraped off. In some of the earlier experiments the hydrogen peroxid was not neutralized.

Lesser Curvature

	H_2O_2 NEUTRALIZED						AVERAGES
Cat							
1. Near cardia	43.0	52.4	44.0	36.2	40.4	35.3	41.9
2.	38.3	42.4	39.5	33.1	39.2	39.4	38.7
3.	30.3	39.0	32.8	35.3	35.4	25.8	33.1
4. Near pylorus	29.6	31.6	36.5	31.9	28.3	23.1	30.2
Dog							
1. Near cardia	23.5	19.5	18.6	26.8	25.2		22.7
2.	17.5	14.4	17.4	15.1	14.7		15.1
3.	17.8	16.4	16.8	11.7	11.4		14.8
4. Near pylorus	8.2	17.6	18.8	11.3	16.0		14.4
	H_2O_2 NOT NEUTRALIZED						AVERAGES
Rabbit							
1. Near cardia	25.0	22.5	25.0	31.5	25.0	28.5	26.3
2.	19.9	22.5	28.3	20.9	28.7	29.0	24.9
3.	18.7	16.2	18.4	15.3	26.2	28.7	20.4
4. Near pylorus	16.0	12.9	19.0	14.0	22.5	25.4	18.3

Greater Curvature

NOTE: For purposes of comparison a test was made also of the catalase in the muscle from the first part of the duodenum.

	H_2O_2 NEUTRALIZED								AVERAGES
Cat									
1. Near cardia	32.6	38.1	57.7	56.4	40.4	49.6	42.8	55.3	44.1
2.	30.9	28.0	48.2	54.8	38.8	35.9	39.4	51.2	40.9
3.	27.4	40.8	43.7	39.1	31.5	41.3	36.8	45.3	38.2
4. Near pylorus	22.4	41.8	44.1	49.2	27.2	32.5	34.3	52.0	37.8
5. Duodenum	24.4	34.7	43.0	46.7	41.0	48.3	32.4	48.6	39.9
Dog									
1. Near cardia	24.0	23.5	26.1	17.4	17.0	19.0			21.2
2.	20.7	22.6	18.8	14.9	15.6	18.5			18.1
3.	29.4	25.9	18.9	16.6	16.2	18.4			20.9
4. Near pylorus	30.5	21.0	10.5	14.8	14.8	22.1			18.9
5. Duodenum	25.2	25.0	23.3	22.2	16.9	21.6			22.4
	H_2O_2 NOT NEUTRALIZED								AVERAGES
Rabbit									
1. Near cardia	17.4	17.0	21.0	17.7	18.8	15.0	15.9	14.7	17.2
2.	14.9	15.6	18.2	11.4	19.9	18.9	16.5	15.0	16.3
3.	16.6	16.2	19.5	17.1	22.0	13.0	15.6	13.6	16.7
4. Near pylorus	14.8	14.8	14.1	14.8	15.9	10.5	18.2	12.4	14.4
5. Duodenum	22.2	16.9	21.3	33.3	18.6	21.9	18.2	15.0	20.9

DISCUSSION

A glance at figure 1 shows the definite gradation in the catalase content from cardia to pylorus. This is most marked along the lesser curvature in the cat and rabbit. The irregularity of the curves in the dog may be due partly to the difficulties encountered in getting perfectly healthy dogs. Animals with definite distemper, large numbers of intestinal parasites or diarrhea had even more irregular curves.

The poorer gradation along the greater curvature in all the animals is to be expected. The remnants of the primitive intestinal tube are along the lesser curvature while a considerable proportion of the greater curvature develops in the embryo as a cecum (3). The curves from the rabbit's stomach conform very closely to what we expected from the previous studies on the latent period, irritability and rhythmicity of the muscle. It is hard to say why in the cat and dog the figures should be higher on the greater curvature, where the rates of contraction were slower. Another thing which is hard to explain is the great difference between the amounts of catalase on the two curvatures near the pylorus in the cat and dog. To be sure, some differences in latent period and irritability were found in different parts of the antrum but when we think of the development and function of this part of the stomach there would seem no need for any differentiation in the muscle along the two curvatures. In order to get a little more light on this subject, estimations were made on equal weights of muscle from the two curvatures and from the anterior and posterior surfaces of the antrum. The following figures from the cat and dog show a striking difference between the catalase content of the muscle on the lesser curvature and that in the rest of the antrum.

	CAT	DOG
Lesser curvature	32.0	21.8
Anterior surface	40.2	32.3
Posterior surface	43.3	33.8
Greater curvature	47.2	43.2

Another interesting point is that the pace-making area near the cardia had a much higher catalase content than that of the pyloric region in which most of the muscular work of the stomach is done. It appears from this that the amount of catalase in a muscle depends not so much upon the amount of work required but upon the speed with which it is to be done. It may be remembered also that the cardia with its weak, shallow but rapid contractions can dominate the rhythm of the antrum with its powerful, deep but slow contractions.

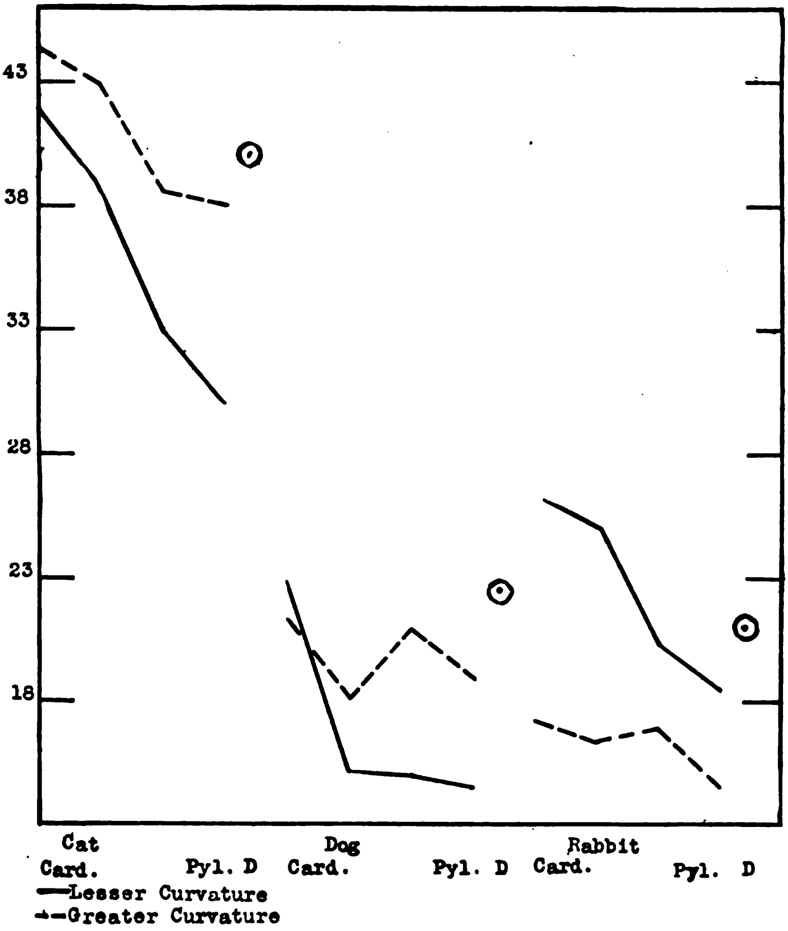


Fig. 1. Ordinates represent amounts of catalase. The three sets of abscissae represent segments of the stomach. The circle and dot represent the catalase content of the duodenal muscle, next to the pylorus. The solid line represents data from segments along the lesser curvature; the broken, from the greater curvature.

If we are correct in assuming that the catalase content of the muscle is an index of its metabolic activity, we may say that there is a metabolic gradient in the stomach similar to that in the intestine. This gradient probably underlies and accounts for the gradients of rhythmicity, irritability and latent period which we believe determine the direction of peristalsis in the stomach.

The muscle in the duodenum adjoining the stomach is very different in its rhythmic activities, latent period, irritability and shape of contraction curve and its high catalase content was to be expected.

The latent period studies on the intact stomach (2) showed that the pyloric ring was a little more irritable than the rest of the antrum. Gaskell has suggested that the muscle in the pyloric and ileo-cecal sphincters has a different phylogenetic origin from that in the rest of the digestive tract (4). This might account for the differences in reaction to adrenalin noted by some observers (5). Keith believes also that there is a rhythmic center in the duodenum about the papilla of Vater (6). It seemed well, therefore, to study the catalase content of a number of adjacent segments in this region. Following are some representative protocols:

	CAT		DOG		RABBIT	
Antrum	23.0	23.8			10.2	10.0
Pyloric ring	20.3	21.0	21.5	20.3	11.7	12.4
Duodenum 1	26.3	27.8	19.2	18.5	38.5	29.0
Duodenum 2	25.6	31.0	20.7	18.6	54.4	49.3
Duodenum 3	32.3	35.2	18.3	21.2	42.8	40.7
Duodenum 4			19.8	19.3		

NOTE: The figures for duodenum in the rabbit represent mucosa and muscle. The other figures are for muscle alone.

These figures do not show much difference between the pyloric muscle and that in the rest of the antrum. They do show, as was rather to be expected, that there is an upward gradation in the first few centimeters of the duodenum before the downward gradation to the ileum begins. Radiologists know that the so-called duodenal cap shows little activity in man and tends to remain filled during digestion. Alvarez has shown also in rabbits that muscle excised from this region has a comparatively poor rhythmicity (7). It appears, then, that the upper end of the intestinal gradient is not at the pylorus but a little below, perhaps about the papilla of Vater. Although Keith's suspicion that there is nodal tissue in this region may be correct, it must be kept in mind clearly that one segment of intestine does not seem to influence the rhythm of the one next to it; the bowel does not pulsate like a heart and the word "pacemaker" must be used with caution. It is a little different in the stomach where the peristaltic rates of the different regions are all subordinated to that of the cardia. It is possible that some of the ripples which run down the intestine arise in the duodenum but records obtained so far suggest that they come over from the stomach.

We hope to take up, in a subsequent paper, the upsets in these gradients found in sickly animals. In all the work on gradients, both in the stomach and in the small intestine, it has been found necessary to make careful note of the condition of the animals as regards friskiness, nutrition, presence of snuffles, distemper, mange, intestinal parasites, etc. The gradients seem to be upset easiest and most frequently in the dog and least frequently in the rabbit. The inability of the rabbit to vomit may be due at least in part to the stability of its gastric and intestinal gradients. An even, steep downward gradient is probably more essential to the welfare of an herbivorous animal, with its rough bulky food, than to a carnivorous animal with its more fluid intestinal contents.

SUMMARY

The catalase content of the gastric muscle is graded downwards from the cardia to the pylorus. This gradient is more marked along the lesser curvature than on the greater. It corresponds pretty well with the gradients of latent period, irritability and rhythmicity previously observed.

The amount of catalase in the muscle seems to depend less upon the amount of work required than upon the speed with which it is to be done.

No peculiarity could be found in the catalase content of the pyloric ring.

Below the pylorus, the catalase content rises rapidly to a point in the middle or lower duodenum from whence the gradient is downward to the colon.

It is believed that these gradients of catalase content represent gradients of metabolism underlying and giving rise to the gradients of rhythmicity and latent period which direct the downward waves of peristalsis.

These gradients are often reversed in sick animals which are vomiting or refusing food.

BIBLIOGRAPHY

(1) **Alvarez and Starkweather**: This Journal, 1918, xlvi, 186.
(2) **Alvarez**: This Journal, 1916, xl, 585; 1916, xli, 321; 1917, xlii, 435.
(3) **Keith and Jones**: Journ. Anat. and Physiol., 1902, xxxvi, 34.
(4) **Gaskell**: The involuntary nervous system, London, 1916, 45.
(5) **Elliott**: Journ. Physiol., 1904, xxxi, 159; Ibid., 1905, xxxii, 416.
Kuroda: Journ. Pharm. Exper. Therap., 1916, ix, 187.
(6) **Keith**: Med. Press and Circular, 1915, xli, 72.
(7) **Alvarez**: This Journal, 1918, xlv, 344.

XV. THE CATALASE CONTENT OF THE MUCOUS MEMBRANE FROM DIFFERENT PARTS OF THE DIGESTIVE TRACT

WALTER C. ALVAREZ AND ESTHER STARKWEATHER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication July 9, 1918

As is now well known, catalase is the ferment which liberates oxygen from hydrogen peroxid. It seems to have something to do with the oxidative processes and a good many writers feel that the catalase content of a tissue can be taken as a fair index of its metabolic activity. We have shown recently that the catalase content of the intestinal muscle is graded from duodenum to colon, i.e., a given weight of duodenal muscle has more catalase than the same weight of ileal or colonic muscle (1). When charted on coördinate paper, the gradients of intestinal rhythmicity, CO_2 production and catalase content were found to parallel one another very closely. There was a definite relation between the catalase content of any one bit of muscle and the degree of activity ordinarily displayed by the segment of intestine from which it was taken.

It seemed worth while, then, to see if we could demonstrate a similar relation between the catalase content of any one piece of mucous membrane and the activity (secretory and absorptive) of that part of the digestive tract. As we will point out later in the discussion, there are marked differences in the amounts of work done by the mucous membrane in different parts of the stomach and bowel. We shall show also that there are differences in the CO_2 production of equal weights of mucous membrane from different parts of the small intestine and colon.

TECHNIC

The technic has been described fully in a previous paper (1). The animals were killed either by a blow on the head or by chloroform and then bled. In dogs and cats the mucosa was peeled off from the muscle; in rabbits it was scraped off. The tissue was ground in a

Stomach—Lesser curvature

							Average
Cat:							
1. Near cardia	37.7	52.7	50.0	46.8	44.9	48.0	46.7
2.	37.4	30.0	50.7	46.2	29.5	31.2	37.5
3.	34.6	26.4	24.5	33.0	29.5	35.9	30.7
4. Near pylorus	32.3	26.1	21.0	30.1	24.4	27.0	26.8
Dog:							
1. Near cardia	21.8	27.4	36.8	34.4			30.1
2.	21.7	23.8	23.4	28.2			24.3
3.	20.8	18.8	20.1	23.6			23.8
4. Near pylorus	8.2	19.2	19.7	21.6			17.2
Rabbit:							
1. Near cardia	48.3	46.3					47.3
2.	41.4	35.3					38.4
3.	38.0	35.2					36.6
4. Near pylorus	35.9	28.8					32.4

Stomach—Greater curvature, upper duodenum

Cat:								
1. Near cardia	30.2	50.8	40.2	42.3	31.7	47.3	44.8	41.0
2.	48.0	36.6	40.0	35.4	33.2	40.5	33.5	38.2
3.	39.3	37.4	32.7	39.7	27.9	31.8	38.2	35.3
4. Near pylorus	29.6	33.5	31.0	40.4	17.4	27.7	26.1	29.4
5. Duodenum, near pylorus	41.4	56.3	58.0	55.6	26.2	32.5	38.6	44.1
Dog:								
1. Near cardia	13.5	18.8	36.3	31.0				24.9
2.	20.8	27.5	33.0	30.7				28.0
3.	24.2	21.6	23.6	25.1				23.6
4. Near pylorus	15.7	20.6	20.3	22.8				19.9
5. Duodenum, near pylorus	32.0	43.5	45.8	36.8				39.5
Rabbit:								
1. Near cardia	43.0	38.2						40.6
2.	38.7	26.0						32.4
3.	18.9	20.8						19.9
4. Near pylorus	21.8	20.2						21.0
5. Duodenum	31.9	29.9						30.9

Intestine

									Average
Cat:									
1. Duodenum	67.2	58.5	84.0	67.0	66.0	62.1	38.6	56.9	67.5
2. Jejunum	56.6	54.7	54.0	49.3	42.1	68.1	24.9	57.2	50.9
3. Middle	51.0	49.3	44.3	43.5	30.7	37.1	33.1	58.2	43.4
4. Ileum	51.8	48.3	35.9	43.2	27.1	40.1	29.4	36.8	39.0
5. Colon	33.7	30.0	37.0	35.0	30.2	34.2	23.9	28.0	31.5
Dog:									
1. Duodenum	44.8	49.1	51.0	46.5	49.6	40.2			46.9
2. Jejunum	32.4	39.4	52.0	47.2	32.4	31.3			39.1
3. Middle	30.7	32.2	45.7	34.2	35.1	30.0			34.7
4. Ileum	32.0	32.5	35.2	35.4	29.3	20.7			30.9
5. Colon	18.2	26.7	28.4	21.4	25.0	15.7			22.6

Colon

Cat:												
1. Upper	45.3	49.4	43.1	46.5	37.6	42.9	52.8	43.7	62.0	45.6	44.2	49.8
2.	50.0	37.4	28.3	39.6	42.5	26.1	48.0	42.5	44.7	59.8	40.7	41.8
3.	38.7	36.2	27.3	35.5	22.6	27.4	39.7	30.5	40.7	48.2	36.5	34.8
4.	24.6	33.9	24.9	34.6	21.8	26.7	37.1	30.7	29.0	38.1	40.3	32.9
5. Lower	27.0	23.5	40.7	31.1	35.0	29.2	58.3	51.4	30.7	27.4	31.5	35.1
Dog:												
1. Upper	23.3	36.2	36.6	42.4	34.9	25.9	26.0					32.2
2.	18.5	18.8	23.3	35.4	30.0	22.3	27.5					25.1
3.	18.6	21.5	30.0	22.3	26.8	21.2	24.9					23.6
4.	19.7	29.9	32.7	27.7	19.3	16.3	22.2					23.9
5. Lower	23.5	15.8	32.6	27.8	29.3	14.4	37.2					25.8

Cecum and Colon

Rabbit.					
1. Tip	42.0	45.4	20.6	32.4	35.1
2.	51.6	50.5	27.3	32.5	40.5
3.	49.8	54.6	29.2	26.8	40.0
4. Base	39.2	34.0	27.0	28.4	32.2
5. Colon	30.6	32.1	22.1	23.6	27.1

mortar and washed into a large test tube. To this were added 15 cc. of a 3 per cent H_2O_2 which had previously been neutralized. The oxygen which was liberated was measured in a burette at atmospheric pressure. By using a shaking machine five such tests on tissue from the same stomach or bowel could be run at the same time and under identical conditions. In the preceeding tables the figures represent cubic centimeters of oxygen. These figures have not been reduced to a common temperature and atmospheric pressure because we are interested only in the gradations observed in the different sets of five readings. In all this work 0.2 gram of mucous membrane was used.

DISCUSSION

A glance at the tables and at figure 1 will show that definite differences in catalase content have been found: differences which are graded very much as they are with the muscle. Incidentally it should be noted that per unit of weight the mucous membrane contains about twice as much catalase as does the muscle. This is what we should expect from the work of Brodie and Vogt (2), who found the oxygen intake and CO_2 output of the mucosa much higher than that of the intestine as a whole.

Let us turn first to an analysis of the differences found in the stomach. It should be remembered that the stomach phylogenetically and embryologically is developed as a bulging in the primitive intestinal tube (3). Originally the dilated lower end of the esophagus joined the pyloric antrum at the incisura angularis. The pyloric antrum is the least modified portion of the primitive tube. The remnant of the esophagus along the lesser curvature forms the gastric canal, a furrow which serves to conduct fluids from the cardia to the antrum. As we should expect, the cells and glands in the mucous membrane in the pyloric antrum, around the cardia and along the lesser curvature, are least differentiated from those in the intestine (4). The acid-forming cells, peculiar to the stomach, are found in the body and fundus of the organ: regions which have developed as a cecum off of the original tube (5). It should be remembered also that the mucosa of the pyloric antrum probably has little function. The antrum serves as a muscular gizzard in which the food is ground up and mixed with juices which trickle down from above.

On turning to the table we find the figures a little larger on the greater curvature than on the lesser. That is what we should expect as the functions of the gastric canal probably do not require great metabolic activity. As we should expect also, there is very little catalase in the mucosa of the pyloric antrum. It is hard to say, however, why there should be so much in the cells around the cardia. One would not expect much digestive activity in that comparatively quiet part of the stomach. It is interesting that the amounts of catalase in the stomach are considerably lower than those in the upper small intestine. This is what we should expect, as the small bowel is the essential organ of digestion. The stomach serves largely as a hopper to pass the food downward in small amounts as needed. A glance at figure 1 will show that the catalase content of a given weight of the gastric mucosa is even less than that of the same weight of colonic mucosa. It should be noted, however, that the gastric mucosa is thick and that in the colon very thin so that the metabolic activity of a given area of the gastric lining is probably much greater than that of the same area in the colon.

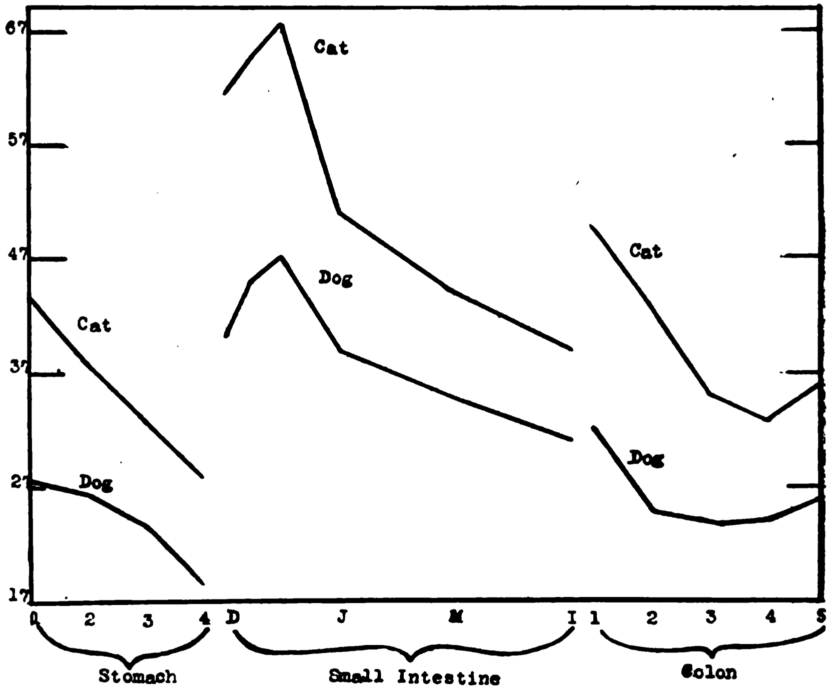


Fig. 1. Shows the gradation in catalase content of the mucosa in different regions. The abscissae represent the regions and the ordinates cubic centimeters of oxygen liberated in fifteen minutes by 0.2 gram of tissue.

On turning to the small intestine it is apparent that there is an abrupt change at the pylorus from a mucous membrane poor in catalase to one rich in catalase. When the data were averaged we found that the duodenal figure in the "greater curvature" tables was greater than that in the "small intestine" table. This seemed to be due to the fact that in the first experiments we had used mucosa next to the pylorus; in the later ones it had been taken from farther down the duodenum. To make sure that the differences were not accidental, the catalase was measured in several adjacent segments as follows:

	CAT			DOG	
Pyloric antrum	34.0	35.8	32.8	17.6	23.3
Duodenum 1	41.5	43.5	37.9	37.5	31.7
Duodenum 2	43.3		38.5	41.1	35.8
Duodenum 3	45.7	45.6	39.5	32.5	37.8
Jejunum	41.6	36.7	36.6	37.7	36.9

It seems from these data that the gradation is generally upwards at first from the pylorus to the middle or lower duodenum, from whence it is downward to the colon. This is as we should expect from what we know of intestinal digestion. All the evidence points to the presence of the greatest activity in the lower duodenum and upper jejunum where the valvulae conniventes and villi are largest and most numerous. A gradation in the metabolic activities of the gut is indicated also by the gradation in the blood supply. Monks (6) has called attention to the local peculiarities of the mesenteric blood vessels, peculiarities which insure a larger blood flow to the jejunum than to the ileum.

We know now that the gastric mucosa absorbs very little; the much thinner and simpler mucous membrane of the colon probably absorbs still less. All those who have studied the question of nutrient enemas agree that little passes through the walls besides sugar, salt and water. This absorption of water seems to be one of the main functions of the colon. In fact, this organ is to the body what a condenser is to a steam engine. There are few glands in the colonic wall and these are concerned mainly with the excretion of mucus. As we should expect, then, the catalase values are low. They are graded downwards from the cecum at first but later they rise toward the anus. It is impossible to say at present just why this last rise should appear.

The cecum in the rabbit plays a more important part in digestion than does the colon and we find its mucosa has a larger catalase content. As we would expect, there is no constant gradation in the figures from different parts of the cecum. Most of the work is probably done by the cells in the middle region of the organ where the bulk of the contents is greatest, and there the catalase content is highest.

On the whole, these results are compatible with the theory that the catalase content of a tissue is an index to its metabolic activity. In order to establish this point a little more definitely, a few estimations were made of the CO_2 production of weighed amounts of mucous membrane taken from five different regions of the cat's bowel. Haas' method was used. It has been described in a previous paper (1). The bits of tissue are sealed into small tubes containing Locke's solution which is stained pink with a trace of phenolsulphonephthalein. The CO_2 given off acidifies and decolorizes the solution. After some time in a water bath, the tubes are graded according to tint. As the mucous membrane does not become active in the Locke's solution the way the muscle does, the decolorization was slower and the gradation less pronounced. In fourteen experiments, however, there was a definite downward tendency from the duodeno-jejunal region to the colon. This gradation is seen also in the following six experiments where the faded tubes were titrated back to their original color. The figures represent drops of n/50 NaOH.

	TIME						
	3 hours	2½ hours	5¾ hours	3½ hours	3½ hours	3½ hours	Average
Duodenum........	10	9	7	10	9	11	9.3
Jejunum..........	10	12	11	13	15	14	12.5
Middle............	8	9	6	10	6	12	8.5
Ileum.............	6	10	6	9	9	9	8.1
Colon.............	8	8	7	8	8	9	8.0

Although the data are few and somewhat irregular in their gradation, they tend strongly to confirm the conclusions arrived at from the catalase studies.

Do these studies throw any light on the predilection of ulcer for certain regions? We believe they do not. According to Burge and others (7), a loss of oxidative power in the mucous membrane will lead to autodigestion by the contained ferments. Magath (8) believes that lumbricoid worms in the intestine owe some of their immunity to trypsin to the presence of considerable amounts of catalase in their integuments. Our studies lend little support to these views because, if conditions in man are similar to those in the cat and dog, the favorite site for ulcer, in the upper duodenum, must have a very high catalase content. The low catalase content of the mucous membrane in the antrum might help the formation of ulcers there but we know that they often are found well up on the lesser curvature where the catalase content should be high.

It is very suggestive that the commonest site for cancer in the digestive tract, the lesser curvature near the pylorus, should have the lowest catalase content. The next lowest place in the animals studied would correspond in man to about the splenic flexure, another common site for cancer. Child (9) has shown with simple forms of life that the progressive differentiation of the cell, with the gradual accumulation of more or less stable structural substances, leads to senescence and death. If the rate of metabolism be greatly slowed by adverse circumstances, such as starvation and drying, these old cells may use up their metaplasm; they dedifferentiate, and in so doing reacquire the faculty of growth and multiplication. Goodpasture (10) has just pointed out how this mechanism, so helpful for the rejuvenation of lower forms of life, can become a menace to the existence of higher forms. It may be, then, that these regions of the mucous membrane with their low rates of metabolism are most senile and therefore most susceptible to cancerous growth. We now have some explanation also for the remarkable immunity of the duodenal mucosa not only to primary cancer but to invasion by growths beginning in the stomach. Pathologists have often wondered why these gastric tumors are always so sharply limited at the pylorus. A glance at figure 1 shows now, as might have been expected, that there are pronounced differences in the metabolism of the cells on the two sides of the sphincter: differences which can, conceivedly, favor or hinder the growth of cancer.

SUMMARY

There is considerable evidence for the view that the catalase content of a tissue is an index of its metabolic activity.

In the gastric mucosa the catalase content is graded downwards from the cardia to the pylorus. The amounts are smaller along the lesser curvature than along the greater. In the small intestine the amounts are graded upwards at first to the middle duodenum and then downwards to the ileo-cecal sphincter.

There is comparatively little catalase in the colonic mucosa and that is graded downwards in the first two-thirds of the tube. The low metabolic activity in this region is against the idea that colonic autointoxication is a common happening. The mucosa of the rabbit's cecum is quite rich in catalase. It shows no constant gradation from one end to the other.

The CO_2 production of equal weights of mucosa is graded from the duodeno-jejunal region to the colon.

These graded results agree quite well with what we know about the digestive activities in the different regions. They do not appear to throw any light on the predilection of ulcer for certain locations.

It is suggestive that the commonest site for cancer has the lowest catalase content. This region with its low rate of metabolism is perhaps most senile and thus most disposed to malignant change.

The abrupt change at the pylorus to a mucous membrane with a high rate of metabolism may easily account for the sharp limitation of gastric cancer usually found at that point.

BIBLIOGRAPHY

(1) Alvarez and Starkweather: This Journal, 1918, xlvi, 186.
(2) Brodie and Vogt: Journ. Physiol., 1910, xl, 135.
(3) Alvarez: This Journal, 1916, xl, 585.
(4) Lansdown and Williamson: Brit. Journ. Surg., 1914, ii, 308.
Bensley: Amer. Journ. Anat., 1902, ii, 122, 128, 133.
(5) Keith and Jones: Journ. Anat. and Physiol., 1902, xxxvi, 34.
(6) Monks: Ann. Surg., 1905, xlii, 549.
(7) Burge and Burge: Journ. Amer. Med. Assoc., 1916, lxvi, 998.
(8) Magath: Journ. Biol. Chem., 1918, xxxiii, 395.
(9) Child: Senescence and rejuvenescence, Chicago, 1915.
(10) Goodpasture: Journ. Med. Research, 1918, xxxviii, 127.

A COMPARISON OF THE ACTIVITY OF EXTRACTS OF THE PARS TUBERALIS WITH EXTRACTS OF OTHER REGIONS OF THE OX PITUITARY

WAYNE J. ATWELL AND CARLETON J. MARINUS

From the Department of Anatomy, Medical School of the University of Michigan

Received for publication July 12, 1918

INTRODUCTION

For some time it has been recognized that the epithelial portion of the hypophysis cerebri consists of two very different parts. One is bulky and glandular while the other is a thin, epithelial layer closely investing the neural lobe. The two parts are separated by a cleft which is the residuum of the original cavity of Rathke's pocket. To the former part Peremeschko (1) has applied the name "Korkschicht" and to the latter "Markschicht," while Herring has termed them "anterior lobe proper" and "pars intermedia" respectively.

Only recently has it been shown that a distinct third epithelial lobe exists, extending nasalwards from the junction of pars intermedia and anterior lobe proper, to surround the infundibulum and to spread out for a variable distance under the brain floor. Because of its close relation to the tuber cinereum in many animals Tilney (2) has named this part the "pars tuberalis."

The pars tuberalis had long been considered as belonging to the pars intermedia. In fact Herring (3) calls it the "tonguelike process of the pars intermedia" and the "extension of pars intermedia round neck of pituitary," although he notes that it is more vascular than that part of the pars intermedia which faces the cleft.

Tilney has shown that the pars tuberalis is distinct histologically and embryologically. One of us—Atwell (4)—has recently given a full account of the development of the pars tuberalis in the rabbit. It is shown to arise from a pair of thickened ridges which are situated at the nasal side of Rathke's pocket and which—contrary to the statements of Tilney--are discernible very early. The two ridges develop into the lateral lobes which later come to lie spread out under the brain floor and which, fusing with each other across the midline, form the pars tuberalis of the adult.

The pars tuberalis has been recognized and its development traced in several vertebrates. Tilney (2) gives a brief account of its development in the cat and the chick; Baumgartner (5) has studied its developmental history in the reptilia; Parker (6) in the marsupialia; and Atwell (4) in the rabbit and (7) in the tailless amphibia. It is well known that the ventral sacs of the hypophysis of certain elasmobranchs have a paired origin and Woerdemann (8) has homologized these sacs with the "lobulus bifurcatus" (pars tuberalis) of the higher vertebrates. The history of the pars tuberalis in the ganoids and the teleosts is practically unknown but it is significant that three different epithelial portions have been recognized. Thus Stendell (9) speaks of a "Hauptlappen," a "Zwischenlappen" and an "Uebergangsteil." Whether the last-named portion is homologous with the pars tuberalis of other vertebrates and whether it arises from a pair of anlagen in these fishes remain to be learned.

The constancy with which the pars tuberalis is present in various vertebrates bespeaks for it, the senior author was led to believe, some distinct physiological function. Accordingly the present study was undertaken with a view to determining what functions, if any, are possessed by it. Since the pars tuberalis has been most often confused in structure with the pars intermedia, our first endeavor has been to compare the activity of extracts prepared from these two parts. This study will not include a comparison of the functions of the pars tuberalis and the anterior lobe proper. Further experimentation along this line is projected.

THE ANATOMY OF THE BOVINE HYPOPHYSIS

In our work we have made use of the pituitary body of the ox, since it is large and easily obtainable. Because the topography of the pars tuberalis has never been described for the hypophysis of the ox, our physiological experiments were necessarily preceded by a rather careful morphological study of the gland. It is extremely important to recognize the extent and relations of the several parts of the organ when "pure" extracts are to be made. We feel that the finer anatomy of the hypophysis too often has been neglected by physiological workers.

The pituitary body as ordinarily received from the packing house has been torn loose from the brain leaving a longer or shorter stalk. Such glands are unfavorable for microscopical study since not all of the pars tuberalis is present. By carefully removing the hypophysis with a considerable portion of the diencephalon and hypothalamus attached for fixation one is able to preserve the attachment of the neural stalk to the infundibulum together with the entire pars tuberalis.

Figure 1 presents a diagrammatic mid-sagittal section through the hypophysis and the adjacent brain floor. The anterior lobe proper (fine stipple) makes up the main bulk of the gland. The pars intermedia (black) is a thin epithelial layer closely applied to the neural lobe. Pars intermedia and anterior lobe proper are separated by the cleft or residual lumen of Rathke's pocket. Near the caudal end of the pars intermedia is a mass of cells resembling the anterior lobe in structure (*c*, fig. 1). This is the cone of Wulzen (10). We are able to confirm the reports of this observer as to the constancy of its presence. In some glands (as shown in our fig. 1) the cone is flattened, in others it is much taller. It is characterized by the presence of numerous acidophile cells such as are so abundant in the anterior lobe proper.

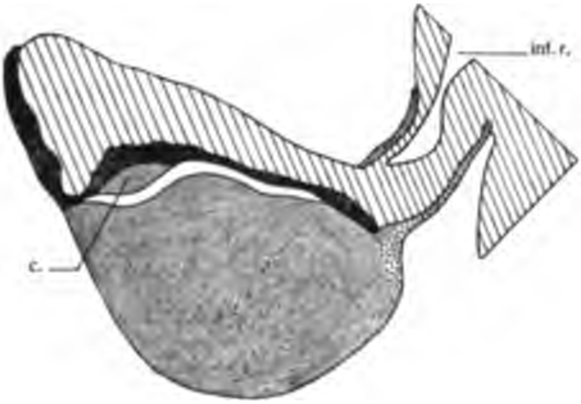


Fig. 1. Diagrammatic median sagittal section of the bovine hypophysis, nasal end at the right. × 4. Lined, neural lobe, neural stalk and brain wall; black, pars intermedia; fine stipple, pars anterior propria; coarse stipple, pars tuberalis; *inf. r.*, infundibular recess; *c.*, cone of Wulzen.

The neural lobe (lined, fig. 1) is attached to the brain floor by a narrow stalk into which the infundibular recess (*inf. r.*) extends for some distance. It is surrounding this neural stalk that the pars tuberalis (coarse stipple, fig. 1) is to be found. It forms a thin layer varying from 0.25 mm. to 1.5 mm. in thickness. While the boundary between the pars tuberalis and the pars intermedia is fairly well defined, the same cannot be said for the junction of the pars tuberalis with the anterior lobe proper. In fact, the pars tuberalis seems to extend for a variable distance over the anterior lobe and to form a cortex for its nasal end.

Histologically the pars tuberalis is readily distinguished from both the pars intermedia and anterior lobe proper. It does not contain the eosinophile cells which are so characteristic of the anterior lobe and it is much more vascular than the pars intermedia. Even in the gross numerous blood vessels may be discerned, disposed in an antero-posterior direction. These are well shown in transverse section in figure 2. The cells of the pars tuberalis are arranged in small blind tubules or acini, the walls of which are composed, generally, of one layer of cells (fig. 3). Occasionally the lumen of an acinus is slightly distended by an accumulation of homogeneous material which takes a faint eosin stain. Many acini do not show a distinct lumen. The cells of the pars tuberalis are lighter than those of the pars intermedia although both have been described as "chromophobe."

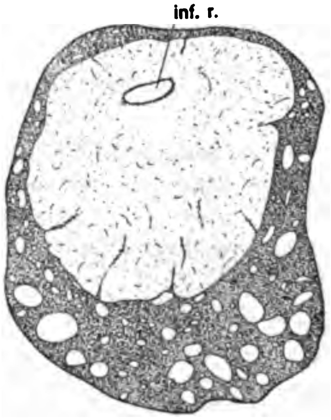


Fig. 2. Transverse section of the hypophyseal stalk. × 20. Neural stalk (light) is in center and is surrounded by the pars tuberalis which shows numerous blood vessels; *inf. r.*, infundibular recess.

Some difference of opinion seems to exist concerning the presence of hyalin bodies in the neural lobe. Lewis, Miller and Matthews (11) state that such bodies do not form a striking picture in the histology of the ox hypophysis. Herring (12) on the other hand finds them very pronounced, some showing the remains of swollen nuclei; "others are in a more advanced stage of alteration and form rounded, oval or irregular colloid masses." We must agree with Herring that hyalin bodies are fairly numerous in the tissues of the neural lobe and that they are also to be found, though less abundantly, in the neural stalk. After routine methods of preparation (formalin fixation, hematoxylin and eosin staining) the hyalin bodies show a faint eosin color and might be very easily overlooked. After a modified Weigert hematoxylin and van Gieson connective tissue stain, however, they are a very dark gray and stand out prominently.

They often contain in their centers darker staining masses. Whether or not these are degenerating nuclei, as claimed by Herring, we are not prepared to state.

The hyalin bodies found in the neural lobe are different chemically from the contents of the acini of the pars tuberalis. This is clearly shown in the Weigert-van Gieson stain. While the hyalin masses stain a dark gray color the material in the acini is colored a light yellow. It seems most likely that the hyalin bodies are derived from the pars intermedia and enter the neural lobe secondarily, as maintained by Herring. We do not believe that the pars tuberalis takes any part in their formation.

THE PREPARATION OF EXTRACTS

For the purpose of comparing the activity of the pars tuberalis and the pars intermedia we endeavored to prepare "pure" extracts which would contain the active principle or principles of each part separately. By using the methods of Lewis, Miller and Matthews (11) and of Herring (12), it was found a comparatively easy matter to obtain the pars intermedia substance in the pure condition. Upon separating the anterior lobe proper from the "posterior lobe" (which consists of neural lobe and the closely investing pars intermedia), by cutting around the margins of the cleft, the surface of the pars intermedia is exposed. Its color is usually distinctive ranging through almost all shades of yellow, orange and brown. There is no marked intermingling of nervous and pars intermedia tissues in the ox (cf. fig. 1). By using a pair of small curved scissors and working where the pars intermedia is the thickest (near the caudal end of the gland) it is possible to snip off small pieces of the yellowish tissue without cutting deep enough to include any of the neural lobe, which is gray. Care was taken to avoid the margins of the cleft and thus we are sure that we prevented contamination from the anterior lobe proper or from the pars tuberalis. The cone of Wulzen was readily recognized and was always avoided.

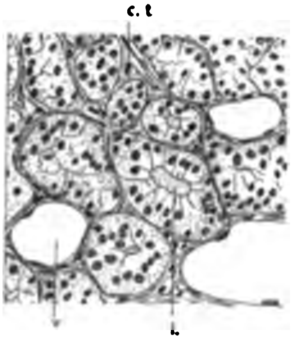


Fig. 3. Section of pars tuberalis of bovine hypophysis. × 300. *l.*, lumen of an acinus distended by a small amount of granular material; *c.t.*, connective tissue; *v.*, one of the smaller vessels.

The separation of the pars tuberalis from the neural stalk which it invests has been supposed to be impossible. For example Herring (12) states, in reference to his "tongue-like process of the pars intermedia" and the neural stalk, "it would be impossible to dissociate the two tissues at this level." After a careful study of the physical properties of the two tissues, however, we have found that they may be separated very satisfactorily. The pars tuberalis is well supported by connective tissue which ramifies throughout its substance (fig. 3). On this account it is tough and not readily broken. The tissue of the neural stalk, on the other hand, is soft and friable.

After some experimenting it was found that the most satisfactory procedure was as follows: the stalk of the gland (the neural stalk and the surrounding pars tuberalis) was cut transversely a sufficient distance anterior to its attachment to the gland to avoid the pars intermedia and the anterior lobe proper. The short cylinder was laid upon a clean glass plate and the pars tuberalis slit its length with fine scissors. It was then found to be a comparatively easy matter to peel off the pars tuberalis as a thin, tough membrane. Any adhering pieces of nervous tissue were removed by gentle scraping with a blunt scalpel. Both the pars tuberalis and the neural stalk tissues were saved.

For the preparation of extracts, then, we had pure pars intermedia, pure tissue of the neural stalk, and pars tuberalis which had, perhaps, a small amount of neural stalk substance adhering but which certainly was uncontaminated by inclusion of any of the pars intermedia or of the anterior lobe proper.

From these tissues we at first prepared extracts by macerating the fresh gland substance in a mortar and then boiling it with dilute acetic acid as described by Roth (13) so that each cubic centimeter of the completed extract represents the water-soluble constituents from 1 mgm. of fresh gland substance. We found this dilution entirely too weak for most of our experiments so that later we prepared extracts having a strength of 10 per cent of the fresh gland. Each cubic centimeter of the stronger extract represents the water-soluble constituents from 100 mgm. of fresh gland substance. Dilutions of this extract were made as required. In certain instances comparisons were made between the action of these extracts and of commercial pituitrin and adrenalin.

THE ACTION OF EXTRACTS

Since the discovery in 1895 by Oliver and Schäfer (14) that extracts of the pituitary body contain an active physiological substance, a number of studies have been made attempting to determine the origin of this substance. Howell (15) soon found that the active principles are resident in the posterior lobe and not in the anterior, an observation which has been confirmed repeatedly. In 1908 Herring (3) showed from histological evidence that the "posterior lobe owes its activity to the investing epithelial layer, the pars intermedia." Crowe, Cushing and Homans (16) concur with Herring in stating that hyalin bodies pass into the third ventricle. Cushing and Goetsch (17) obtained a pressor effect by injecting concentrated human cerebro-spinal fluid into rabbits. Carlson and Martin (18), however, were not able to obtain a pressor effect from the cerebro-spinal fluid of normal dogs.

Lewis, Miller and Matthews (11) made extensive studies upon the activity of extracts from various parts of the ox pituitary. From extracts of pure pars intermedia they obtained a pressor effect on dogs. These authors tested extracts made from the stalk of the gland or "infundibular walls" and failed to obtain pressor effects. On this account they doubt the passage of secretion from the neural lobe into the third ventricle. Biedl was able to confirm the observation that a pressor substance is yielded by extracts of the pure pars intermedia, but he failed to obtain constantly such effects from the neural lobe. Herring found that the pars intermedia of the ox pituitary yields a substance which stimulates uterine contraction and increases the secretion of milk but which exercises little if any pressor effect in the cat. Extracts of the neural lobe were found "to yield, in relatively larger amount, the material which stimulates the uterus and acts as a galactagogue." In addition, extracts of the neural lobe produce a rise of blood pressure in the cat.

The use of pituitary extracts to stimulate peristalsis in cases of post-operative intestinal stasis has become quite general since its suggestion by Bell and Hick (19). Duffy (20) has recently reviewed the literature and strongly recommends pituitary extract for the relief of intestinal paresis. Certain authors, however, have noted that inhibition of the normal intestinal rhythm may be produced by pituitary extracts. Bayer and Peter (21) using 0.4 to 5.0 cc. of pituitrin in 15 to 20 cc. Ringer's solution obtained marked slowing and inhibition of the rhythm of segments of rabbit intestine. Shamoff (22) used a much greater dilution and obtained inhibition of activity on the isolated intestinal segment. When 1 mgm. of dried posterior lobe substance was shaken up in 5 cc. of Ringer's solution and later the clear fluid decanted and added to the Ringer's solution containing the intestinal segment, a distinct inhibitory effect was observed. A similar effect was obtained with certain commercial pituitary extracts in a dilution of 1:5000. Hoskins (23) observed inhibition of peristalsis in the intact intestine of the dog following intravenous injection of pituitrin, and also following injection of saline extracts prepared from the desiccated gland. Roth (13) obtained inhibition of the intestinal segment of the rabbit in pituitrin diluted 1:40. He ascribes the inhibitory effect to the presence of chlorbutanol, which is used as a preservative in this pituitary extract.

Our experiments include tests made to determine what action, if any, is possessed by the pars tuberalis of the ox pituitary upon the isolated uterine segment, the isolated intestinal segment, and what action upon the blood pressure of the rabbit and of the dog.

a. *Upon the isolated uterine segment.* The effect of extracts of the pars tuberalis and of the pars intermedia was tested upon the isolated uterine segment of young guinea pigs. The method described by Dale and Laidlaw (24) was followed. The uterus of a virgin guinea pig weighing 300 to 350 grams was employed. It was suspended in about 100 cc. of oxygenated Locke's solution and maintained at 38 to 39°C. Measured quantities of the extracts to be tested were added to the Locke's solution and allowed to act until a maximum contraction had been obtained. This was ordinarily not longer than thirty seconds. After washing with fresh Ringer's at least five minutes was allowed before another test was attempted.

The extracts used were the weaker (Roth's "standard"), each cubic centimeter representing the water-soluble principles from 1 mgm. of the fresh gland. In a preliminary test 2 cc. of pars intermedia extract elicited a contraction of 35 mm., while 2 cc. of pars tuberalis extract gave a contraction of only 5 mm. The record of one series of readings obtained consecutively is given in table 1, page 84.

Computing from this table, the average contraction value in millimeters for each cubic centimeter of pars intermedia extract is 33; while the same average value per cubic centimeter of pars tuberalis extract is 4.45 mm. Thus we find that extracts of the pars tuberalis are very much inferior to extracts of the pars intermedia in producing contraction of the isolated uterus of the guinea pig. By computing the relative activity of the two extracts it will be seen that the pars intermedia extract is approximately seven times as powerful as the pars tuberalis extract.

b. Upon the isolated intestinal segment. The several pituitary extracts were tested for their effects upon the isolated intestinal segment of the rabbit. Commercial pituitrin was also tested in a number of dilutions. The rabbit was stunned by a blow, then bled, this procedure being chosen to avoid the inhibitory effect produced by an anesthetic. The intestinal segment was suspended in warm oxygenated Ringer's solution in the same apparatus used for the uterine segment experiments.

We have not observed inhibition of intestinal rhythm by the action of pituitary extracts except in rather weak dilutions. Comparatively strong extracts increased the activity of the segment. When 0.5 cc. of 10 per cent extract of pars intermedia is added to the segment contracting spontaneously in 100 cc. Ringer's solution, a slowing of the rhythm and a strengthening of the contractions is produced. A similar effect was caused by the addition of 0.5 cc. of pituition, except that the effect was more marked.

TABLE 1

Uterus test

TIME	EXTRACT TESTED 1:1000	HEIGHT OF RESULTING CONTRACTION	
		Pars intermedia	Pars tuberalis
		mm.	mm.
4:47	5 cc. pars tuberalis		20
4:53	5 cc. pars tuberalis		22
4:59	5 cc. pars tuberalis		20
5:05	1 cc. pars intermedia	34.0	
5:10	0.5 cc. pars intermedia	15.5	
5:15	5 cc. pars tuberalis		27

Inhibition of intestinal activity was obtained in certain cases by the use of very dilute extracts. Like Shamoff, we find that the inhibitory effect is not a constant phenomenon. On one occasion a clear-cut case of inhibition was obtained by shaking 1 mgm. of dried pars intermedia with 5 cc. of Ringer's solution and adding the supernatant fluid to the test bath. The similar use of 1 mgm. of dried pars tuberalis failed to produce an inhibitory effect.

In figure 4 is shown the effect produced by *A*, 0.2 cc. of 1: 1000 adrenalin chloride; *B*, 5 cc. of Ringer's solution shaken up with 1 mgm. dry pars intermedia; and *C*, 5 cc. Ringer's shaken up with 1 mgm. dry pars tuberalis.

c. Upon the blood pressure. 1. Of the rabbit. A female rabbit weighing 3.6 kilos was anesthetized by paraldehyde *per os;* the right jugular vein was exposed for the administration of extracts and changes in blood pressure were recorded from the left carotid. The extracts tested in this experiment were of the weaker variety (1: 1000 of the fresh gland). The intravenous injection of 1 cc., 2 cc. or 5 cc. of 1: 1000 extract of pars tuberalis failed to call forth any pressor effect. The administration of 2 cc. of 1: 1000 extract of pars intermedia, however, elicited a slight rise of pressure while 5 cc. of the same extract produced

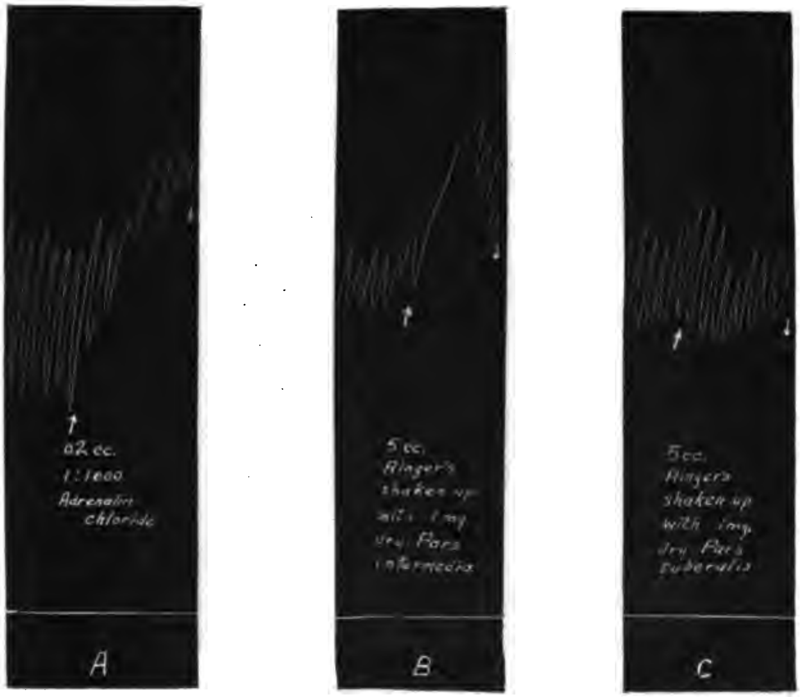


Fig. 4. To show the effect of various extracts upon the isolated intestinal segment of the rabbit contracting spontaneously in oxygenated Ringer's solution. Downstroke represents contraction.

a well-defined rise (fig. 5). The pressor effect was of comparatively short duration.

We find, then, that an extract of the isolated pars intermedia of the ox pituitary has a distinct pressor effect upon the blood pressure of the rabbit when injected in amounts as small as the equivalent of 2 to 5 mgm. of the fresh gland substance. Equal amounts of the pars tuberalis fail to produce a rise in pressure.

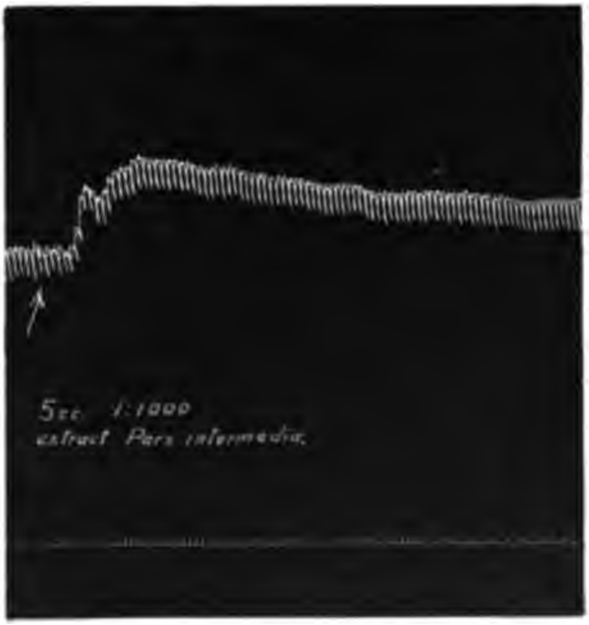


Fig. 5. To show the effect upon the blood pressure of the rabbit following intravenous injection of pure pars intermedia extract. Time is in seconds.

2. Of the dog. Intravenous injection of 0.3 cc. pituitrin made up to 5 cc. in salt solution produced a typical prolonged rise of pressure preceded by a brief depression. After about thirty minutes 0.6 cc. of 10 per cent extract of pars intermedia in 5 cc. salt solution called forth a strikingly similar reaction consisting of a preliminary depression and a subsequent prolonged rise.

In two experiments the effects of intravenous injections of commercial pituitrin, extract of pars tuberalis and extract of neural stalk were compared. The two experiments gave similar results and only the data from experiment 2 will be presented in detail.

A male dog weighing 20 kilos was anesthetized by intraperitoneal injection of chloretone in olive oil. The extracts were introduced into the left femoral vein and the blood pressure record was obtained from the left carotid artery. The extracts of pars tuberalis and of neural stalk used in this experiment were equivalent to 10 per cent of the fresh gland; that is, each cubic centimeter of the extract represented the water-soluble constituents of 100 mgm. of fresh gland substance. The pituitrin used was diluted to one-tenth the strength of commercial pituitrin.

TABLE 2

Blood pressure in the dog

NO.	DOSE	EXTRACT	RISE OF BLOOD PRESSURE IN MILLIMETERS OF MERCURY
	cc.		mm.
A	2	Pars tuberalis	6.5
B	4	Pars tuberalis	14.5
C	2	Neural stalk	36.5
D	2	Pars tuberalis	6.5
E	1	Pituitrin $\frac{1}{10}$	16.0
F	1	Neural stalk	19.0
G	4	Pars tuberalis	12.0
H	1	Neural stalk	20.5
I	4	Pars tuberalis	12.0
J	1	Pituitrin $\frac{1}{10}$	18.0

Summary from table 2. Averages of blood pressure reactions (rise of blood pressure in millimeters)

PITUITRIN 1 cc. ($\frac{1}{10}$)	PARS TUBERALIS 2 cc.	PARS TUBERALIS 4 cc.	NEURAL STALK 1 cc.	NEURAL STALK 2 cc.
16.0	6.5	14.5	19.0	36.5
18.0	6.5	12.0	20.5	
		12.0		
Av. 17.0	6.5	12.8	19.7	36.5

Average pressor value in millimeters per cubic centimeter of extract

PITUITRIN $\frac{1}{10}$	PARS TUBERALIS	NEURAL STALK
17.0	3.2	19.0

Computing from the above data it is seen that a 10 per cent extract of neural stalk is somewhat more powerful in raising blood pressure in the dog than is an equal amount of 10 per cent pituitrin. Extracts of the pars tuberalis are very inferior to those of the neural stalk in pressor effect. Numerically expressed, extracts of the neural stalk are almost six times as powerful as extracts of the pars tuberalis.

DISCUSSION

Our observations have shown that extracts of the pars tuberalis are very inferior to extracts of the pars intermedia in producing contraction of the isolated uterine segment and likewise are inferior to extracts of the neural stalk in raising the blood pressure of the dog. Two things especially are to be noted. First: the pressure curves produced by these three extracts are similar, indicating that we are dealing with one substance or at least with the same group of substances in all cases. Second: the method of preparation of the extracts makes it certain that the pars intermedia and neural stalk extracts were pure, but it is highly probable that the pars tuberalis extract was contaminated by the inclusion of small amounts of the neural stalk.

It is our belief that the active effects shown by extracts of the pars tuberalis are due to the inclusion of neural stalk substance. In other words we believe that if an extract of the pars tuberalis could be obtained in an absolutely pure condition it would not be capable of the active effects which we have noted.

Lewis, Miller and Matthews (11) failed to obtain a pressor effect from extracts of the "infundibular walls" and so concluded that there is a distinct break in the path of secretion between the neural lobe and the third ventricle. At the time these authors performed their experiments the existence of the pars tuberalis as a distinct lobe had not been recognized. It has been our observation that in glands received from the packing house there is considerable retraction of the soft neural tissue in the centre of the stalk, leaving a false "infundibulum" the walls of which are composed mainly of the pars tuberalis. This tissue is very feeble in producing pressor effects, as we have shown. In the preparation of our neural stalk extract we used only careful isolated neural tissue and have obtained constantly a striking pressor effect on the dog. It is believed that this observation removes the objection made by Lewis, Miller and Matthews against the possibility that secretion may pass from the neural lobe into the third ventricle *via* the neural stalk.

Biedl (25) states that extracts of pure neural tissue of the hypophysis do not produce a pressor effect, but Herring has controverted this statement since he has been able to obtain constantly a rise of pressure in cats with such extracts. Our observations on the effects produced by extracts of the isolated neural stalk lend support to the contentions of Herring.

Lewis, Miller and Matthews and likewise Biedl obtained a marked rise of blood pressure in the dog by intravenous injection of pure pars intermedia extracts. Herring, however, failed to obtain similar effects in the cat. We have obtained strong pressor effects in the dog, thus confirming the observations of Lewis, Miller and Matthews and of Biedl. Of course, as Herring suggests, the difference of results obtained may be due to differences in reaction peculiar to the species of animal used. We have not tested the effect of pars intermedia extract on the cat but we are able to state that in the rabbit an extract representing as little as 5 mgm. of fresh pars intermedia of the ox causes a distinct rise of pressure.

The depression or complete inhibition of intestinal activity which sometimes follows administration of pituitary extracts is as yet an imperfectly understood phenomenon. In reviewing the results of those few observers who have obtained such depression one is struck by the wide variance of concentration in the extracts used. Of authors who record depression with the isolated intestinal segment Bayer and Peter (21) and Roth (13) used comparatively concentrated solutions, while Shamoff (22) and ourselves have used very dilute solutions. Hoskins (23) alone has recorded inhibition of activity in the intact intestines.

Bayer and Peter obtained inhibition by the use of from 2 to 5 cc. pituitrin in 15 to 20 cc. Ringer's solution, and Roth observed inhibition when the intestine segment was suspended in pituitrin diluted 1:40. Roth ascribes the inhibitory effect to the chlorbutanol present in this pituitary extract since he obtained a similar inhibition with a solution containing an equivalent amount of chlorbutanol alone. This is probably the correct explanation when inhibition is obtained in such enormous concentrations. But this certainly does not apply to the inhibitions recorded by Shamoff who did not use ordinary pituitrin and who obtained only slight depression with "pituitrin A," while he obtained more distinct inhibition with other commercial preparations as well as with extracts prepared from fresh and dried glands.

The only inhibition of clinical interest is that resulting from the weaker dilutions since it is manifestly impossible to attain a concentration as great as 1:40 for instance, in the living animal. Further observations on the intact intestine are necessary to determine whether such inhibitory effects constitute an indication against the use of pituitary extracts for the relief of intestinal stasis.

SUMMARY AND CONCLUSIONS

1. A "pars tuberalis" may be distinguished in the bovine hypophysis. It differs histologically from both the pars intermedia and the pars anterior propria.

2. The pars tuberalis may be separated fairly definitely from the neural stalk which it invests and may be obtained in sufficient quantity for the preparation of extracts.

3. The extract of pars tuberalis so obtained is doubtless contaminated by the presence of small amounts of neural stalk substance but it is certain that there is no admixture of tissue from the pars intermedia or from the pars anterior propria.

4. Extracts of the pars tuberalis are very inferior to extracts of the pars intermedia in producing contractions of the isolated uterine segment and are likewise inferior to extracts of the neural stalk in raising the blood pressure of the dog. Extracts of the pars tuberalis equivalent to 5 mgm. of fresh gland substance failed to produce a rise of blood pressure in the rabbit.

5. It is believed that the active effects displayed by extracts of the pars tuberalis are due to an inclusion of some of the neural stalk substance during the preparation of the extract. It would seem improbable that the pars tuberalis is responsible for the origin of active principles which increase uterine contraction or raise the blood pressure.

6. An extract of the pure pars intermedia of the ox is capable of producing a strong pressor effect when injected intravenously into the dog. In this we confirm the observations of Lewis, Miller and Matthews and of Biedl. We are able to add that an extract of pure pars intermedia representing 5 mgm of the fresh gland substance produces a distinct rise of blood pressure in the rabbit.

7. An extract of the pure neural stalk is fairly powerful in producing a rise of blood pressure in the dog. This removes one objection to the possibility that the secretion of the pars intermedia passes into the neural lobe and then, *via* the neural stalk, into the third ventricle.

We wish to express our sincere thanks to Prof. C. W. Edmunds for the use of apparatus in the pharmacological laboratories and for very generous personal assistance in the animal experiments. We wish also to thank the research laboratory of Parke, Davis & Company for numerous courtesies shown us during the course of these studies.

BIBLIOGRAPHY

(1) **Peremeschko**: Arch. f. path. Anat. u. Physiol. (Virchow), 1867, xxxviii, 329.
(2) **Tilney**: Internat. Monatschr. f. Anat. u. Physiol., 1913, xxx, 258.
(3) **Herring**: Quart. Journ. Exper. Physiol., 1908, i, 121.
(4) **Atwell**: Amer. Journ. Anat., 1918, xxiv, no. 3.
(5) **Baumgartner**: Journ. Morph., 1916, xxviii, 209.
(6) **Parker**: Journ. Anat., 1917, li.
(7) **Atwell**: Anat. Rec., 1918, xv, no. 2.
(8) **Woerdeman**: Arch. f. mikr. Anat., 1914, lxxxvi, 198.
(9) **Stendell**: Die Hypophysis cerebri, 1914, Teil viii in Oppel's Lehrb. d. vergl. mikros. Anat. d. Wirbeltiere.
(10) **Wulzen**: Anat. Rec., 1914, viii, 403.
(11) **Lewis, Miller and Matthews**: Arch. Int. Med., 1911, vii, 785.
(12) **Herring**: Quart. Journ. Exper. Physiol., 1914, viii, 245.
(13) **Roth**: Bull. 109, Hyg. Lab., Washington, 1916.
(14) **Oliver and Schäfer**: Journ. Physiol., 1895, xviii, 277.
(15) **Howell**: Journ. Exper. Med., 1898, iii, 245.
(16) **Crowe, Cushing and Homans**: Johns Hopkins Hosp. Bull., 1910, xxi, 151.
(17) **Cushing and Goetsch**: This Journal, 1910, xxvii, 60.
(18) **Carlson and Martin**: This Journal, 1911, xxix, 64.
(19) **Bell and Hick**: Brit. Med. Journ., 1909, i, 777.
(20) **Duffy**: New York Med. Journ., 1915, ci, 72.
(21) **Bayer and Peter**: Arch. f. exper. Path. u. Pharm., 1911, lxiv, 204.
(22) **Shamoff**: This Journal, 1916, xxxix, 268.
(23) **Hoskins**: Journ. Amer. Med. Assoc., 1916, lxvi, 733.
(24) **Dale and Laidlaw**: Journ. Pharm. Exper. Therap., 1912, iv, 75.
(25) **Biedl**: Innere Sekretion, 1913, 2 Auf., ii Teil.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS

VIII. The Effects of Quinine on the Production of Egg Yolk and Egg Albumen

OSCAR RIDDLE and CARL E. ANDERSON

From the Station for Experimental Evolution, Cold Spring Harbor, Long Island, N.Y.

Received for publication July 13, 1918

It is well known that small quantities of quinine produce in man and mammals a marked change in the metabolism of the nitrogenous constituents of the tissues. An early increase of nitrogenous bodies found in the urine is soon followed by a prolonged period of markedly diminished amounts of these substances, especially urea and uric acid. That nitrogenous food is not dissipated so rapidly but is stored in the body in some form, was shown by v. Noorden (1) in individuals kept under constant diet. In these cases the absorption of nitrogen was unchanged, its excretion was diminished and the diminution was continued for two or more days after the dosage with quinine was stopped. Our knowledge of the effect of quinine on the oxidation of the tissues includes some observations which are not easily harmonized. It has been stated that in relatively undifferentiated protoplasm—in the simpler protozoa (2) and in the ovum (3) of echinoderms—quinine retards oxidation. On these same forms, however, quinine was recognized as a general protoplasmic poison and it seems to us probable that the observed reduction of the oxidations was essentially a phase of the poisoning by the drug. In the differentiated tissues of the healthy human adult, where the methods of measuring the rate of oxidation have been much more adequate, the results have shown that there is no diminution of oxygen consumption and no decrease in the output of carbon dioxide under quinine.

Conditions present in birds offer unusual advantages for first, a study of the nitrogen conserving action of quinine in the case of a gland (the oviduct or uterus) whose sole product or secretion (egg albumen), in so far as the egg is concerned, is almost exclusively protein; second, the similar action of quinine on the utilization of nitrogen in the last and rapid stages of growth of the ovum (yolk); third, the possibilities of testing the developmental capacities of such modified ova. Concerning the latter it may be said that the ova of pure species of doves and pigeons have been found by one of us (4) to show two kinds of eggs in respect of their metabolic and size differences and that these two levels of metabolism (which involve size as one known differential) have been correlated each with a different sex of the prospective individuals which are developed from these two kinds of eggs.

The present paper is concerned partly with the effects of the quinine on the utilization of protein by the oviduct and the ovum and partly with a demonstration of a definite change in the size of the ova developed under quinine. These ova (yolks) are smaller. The sex data derived from the incubated eggs of birds dosed precisely as were those of this series are being accumulated. Those data must, however, be considered in a later publication. Besides showing a decreased size of the ova, which decrease continues for several days after the dosage is discontinued, the data herewith given supply evidence that quinine causes a reduction from the expected or normal amounts of egg albumen (white of egg). It is highly probable that after a few weeks the amount of albumen secreted is increased considerably beyond the normal, certainly more than was present in the pre-dosing period.

MATERIAL AND METHOD

The birds used were all blond or white ring-doves (*Streptopelia risoria, St. alba*) or hybrids of these two forms. Only freely laying females, which with their male mates occupied separate pens, were dosed. Quinine sulfate in gelatin coated pills was used. The size of the dose was ¼ grain at first and ½ grain later. The date of change of dosage was April 6 except when otherwise indicated in the tabulated records. These amounts were given twice daily, at about 9.00 a.m. and 6.00 p.m., except for a period of four days following the laying of the first egg of each pair of eggs. It was thought best not to handle or disturb the females at a time when this might cause the eggs to be broken or when the beginning of growth of the succeeding pair of yolks might be prevented. A pair or clutch of eggs was obtained each eight or ten days from most of the birds.

These relatively tame doves could practically always be lifted from their perches and the pellet dropped into the back of the mouth without arousing special struggle or excitement. As a control, however, for the possible effect of handling, six of the eleven dosed birds were caught and blank-dosed twice daily from the time of the last dosage with quinine till June 16. The birds thus blank-dosed gave results wholly similar to those not thus treated. The effects observed are certainly therefore the effects of the quinine.

All eggs were examined as soon as laid—within five minutes to three hours of laying. The fresh eggs from the quininized birds were weighed and then steamed for eight minutes to coagulate the yolk and the albumen. The yolks were then immediately freed and weighed. The albumen was not separately weighed for the reason that this water-rich substance cannot be separated from both shell and yolk without an inaccuracy caused by the evaporation of water. Since the amount of shell is a relatively constant[1] and small proportion of the total weight, and showed no obvious variation in dosed[2] and not-dosed eggs, such separate weighing of the albumen is not necessary to a demonstration of the large differences observed.

Twelve yolks obtained from quininized doves were burned in a bomb calorimeter. The yolks which serve as a control for those twelve determinations were not obtained from the same birds, but from other untreated birds of similar origin and laid at a similar season of the year.

PRESENTATION OF DATA

The changes in egg size and yolk size due to quinine in all of the treated birds are indicated in tables 1 and 2. The weight averages of the six to eight eggs and yolks which immediately preceded those produced under dosage are given for each bird. Similarly the average weight of the entire group of eggs produced under dosage is next given. This group shows a pronounced decrease in egg size and yolk size in all of the treated birds. The first egg or pair of eggs produced after the quinine dosage was discontinued shows a further reduction of egg size in one-half of the group; for yolk size this further reduction occurred in every case. Following this pair of eggs, however, the data show a progressive increase in egg size and in yolk size. At six to seven weeks after treatment was discontinued the normal egg size was exceeded in six birds and was not yet attained in four birds; for one bird the data are missing, since this bird produced no eggs after the treatment was stopped. The normal yolk size at six or seven weeks had not, however, been attained in any instance. The data are available to the tenth week and the results are the same. In most cases the yolks still re-

---

[1] The smaller the egg the greater the proportion of shell in relation to total egg size. This means that the amount of albumen in the smaller quininized eggs is even less than is indicated by the comparisons later to be made of yolk size with total egg size.

[2] Among the eggs laid by some of these birds several weeks after the dosing was discontinued a probably unusual number of eggs with weak or imperfect shells has been found. It is yet too early to be sure that this was a result of the previous treatment with quinine.

TABLE 1

The effect of quinine sulfate on egg size and yolk size in ring-doves

NUMBER OF BIRD	DURATION OF DOSAGE	PERIODS AS RELATED TO DOSAGE	NUMBER OF EGGS	AVERAGE WEIGHT	
				Eggs	Yolks
152	February 15 to April 30...	Before	6	8.414	1.869
		During	9	*7.711*	*1.627*
		First after	2	7.935	1.547
		Later	6	8.449	1.591
		Last	5	8.421	1.733
A347	February 19 to April 30...	Before	8	8.419	1.882
		During	10	*7.336*	*1.649*
		First after	2	6.572	1.349
		Later	4	6.934	1.593
		Last	4	7.425	1.730
903	February 23 to April 30...	Before	8	8.415	2.112
		During	8	*7.976*	*2.001*
		First after	2	8.209	1.961
		Later	8	8.388	1.899
		Last	4	8.852	1.918
24	April 5 to April 30........	Before	7	8.198	1.884
		During	0		
		First after	2	7.563	1.358
		Later	4	7.985	1.594
		Last	4	7.987	1.667
962	April 5 to April 30........	Before	8	9.073	1.982
		During	3	*8.163*	*1.754*
		First after	2	7.739	1.617
		Later	4	8.628	1.701
		Last	4	8.841	1.844

TABLE 2

The effect of quinine sulfate on egg size and yolk size in ring-doves

NUMBER OF BIRD	DURATION OF DOSAGE	PERIODS AS RELATED TO DOSAGE	NUMBER OF EGGS	AVERAGE WEIGHT	
				Eggs	Yolks
A174	February 14 to April 30...	Before	8	8.711	2.077
		During	9	*7.354*	*1.811*
		First after	2	7.163	1.560
		Later	6	8.012	1.766
K459	February 19 to April 30...	Before	4	7.411	1.781
		During	2	*6.744*	*1.567*
		First after	2	6.270	1.253
		Later	6	7.361	1.436
		Last	4	7.786	1.534
A 22	March 23 to April 29......	Before	8	8.934	2.109
		During	2	*7.190*	*1.644*
		First after	1	7.504	1.513
		Later	6	9.078	1.958
		Last	6	9.267	1.961
E106	April 5 to April 30........	Before	6	8.417	1.901
		During	6	*8.009*	*1.765*
		First after	2	7.807	1.617
		Later	6	8.486	1.725
		Last	8	8.977	1.833
B508	April 5 to April 30........	Before	6	8.551	1.738
		During	1	*7.481*	*1.339**
E 97	April 8 to April 30........	Before	7	8.053	2.097
		During	2	*7.196*	*1.723*
		First after	2	7.459	1.422
		Later	6	7.806	1.637
		Last	4	8.093	1.743

* No later eggs to July 7.

mained much below normal size. The occurrence of excess of total egg size accoompanied by deficient yolk size in this post-treatment period is practically conclusive evidence for an excessive or supernormal production of egg albumen in this period.

The individual egg weights and yolk weights for the several abovementioned periods, as related to dosage, are given for four of the eleven birds in the left-hand columns of tables 3 and 4. It can there be seen

TABLE 3

Detailed records of egg size and yolk size under dosage with quinine. Also showing that in yolks of comparable size from the same bird the dosed yolks (columns 4, 5) are associated with smaller amounts of albumen (+ shell) than the earlier yolks (not dosed, columns 6, 7). For yolks produced long after dosage (rows designated "Later") the above rule is probably reversed.

NUMBER OF FEMALE	PERIOD WITH REFERENCE TO DOSAGE	DATE OF EGG	EGG WEIGHT	YOLK WEIGHTS Dosed	YOLK WEIGHTS Not dosed	EGG WEIGHT
152	During (February 15 to April 30).........................	3/7	7.592	1.621	1.615	8.095
		3/9	8.380	1.838	1.844	8.378
		3/21	7.578	1.598	1.595	7.968
		3/23	8.275	1.802	1.802	7.860
		4/1	7.792	1.578	1.590	8.040
		4/3	8.492	1.768	1.753	8.110
		4/11	7.038*	1.319*	1.330	7.530
		4/13	7.502	1.690	1.700	8.070
		4/24	6.763	1.428	1.450	7.070
	First after....................	5/7	7.565	1.420	1.498	7.828
		5/9	8.305	1.673	1.660	8.400
	Later.........................	5/17	8.116	1.486	1.498	7.828
		5/19	8.545	1.585	1.595	7.968
		5/25	8.308	1.465	1.450	7.070
		5/27	9.027	1.785	1.802	7.860
		6/3	7.950	1.452	1.450	7.070
		6/5	8.748	1.773	1.753	8.110
		6/11	7.763	1.444	1.450	7.070
		6/13	8.786	1.740	1.753	8.110
903	During (February 23 to April 30).........................	2/27	7.820	1.980	2.005	8.035
		3/1	8.551	2.255	2.238	7.563
		3/25	7.748	1.970	1.930	8.140
		3/27	7.715	1.912	1.910	7.950
		4/5	7.810	1.945	1.923	8.215
		4/7	8.708	2.175	2.167	8.662
		4/20	7.671*	1.900*	1.890	8.430
		4/22	7.785	1.950	1.930	8.140
	First after....................	5/7	7.682	1.790	1.725	7.740
		5/9	8.735	2.132	2.140	8.445
	Later.........................	5/16	7.921	1.815		
		5/18	9.300	2.255	2.238	7.563
		5/25	8.206	1.870	1.910	7.950
		5/27	9.202	2.305	2.309	9.022
		6/2	7.750	1.618		
		6/4	8.321	1.710	1.725	7.740
		6/11	7.880	1.690	1.725	7.740
		6/13	8.525	1.930	1.930	8.140

* First egg produced after increasing the dose of quinine.

† Dates apply only to the eggs whose weights are given in the adjoining column.

TABLE 4

Detailed records of egg size and yolk size under dosage with quinine. Also showing that in yolks of comparable size from the same bird the dosed yolks (columns 4, 5) are associated with smaller amounts of albumen (+ shell) than the earlier yolks (not dosed, columns 6, 7). For yolks produced long after dosage (rows designated "Later") the above rule is probably reversed.

NUMBER OF FEMALE	PERIOD WITH REFERENCE TO DOSAGE	DATE OF EGG	EGG WEIGHT	YOLK WEIGHTS†		EGG WEIGHT
				Dosed	Not dosed	
A347	During (February 19 to April 30)	2/28	6.860	1.720	1.702	7.864
		3/2	7.143	1.625	1.633	7.652
		3/13	7.677	1.741	1.745	7.748
		3/15	7.863	1.795	1.775	8.240
		3/22	7.298	1.515	1.539	7.715
		3/24	7.960	1.868	1.850	8.440
		3/31	6.918	1.415		
		4/2	7.708	1.722	1.728	8.692
		4/9	7.095*	1.518*	1.539	7.715
		4/11	6.828	1.567	1.560	7.642
	First after	5/16	6.259	1.314		
		5/18	6.885	1.385		
	Later	5/26	6.999	1.475	1.480	7.480
		5/28	7.556	1.701	1.702	7.864
		6/6	6.645	1.615	1.633	8.765
		6/8	6.535	1.580	1.488	7.380
		6/25	6.965	1.565	1.560	7.642
		6/27	7.895	(1.870)	1.850	8.440
		7/4	7.357	1.600	1.560	7.642
		7/6	7.684	1.885	1.885	8.060
E106	During (April 5 to April 30)	4/7	8.835*	1.872*	1.877	8.068
		4/9	8.165	1.938	1.954	9.183
		4/16	8.038	1.708	1.771	8.800
		4/18	7.540	1.865	1.877	8.068
		4/25	7.890	1.580	1.589	8.400
		4/27	7.588	1.625	1.620	8.277
	First after	5/4	7.530	1.488	1.508	8.025
		5/6	8.083	1.746	1.778	8.172
	Later	5/12	8.415	1.623	1.620	8.277
		5/14	8.773	1.795	1.795	8.820
		5/20	8.470	1.680	1.695	8.365
		5/22	8.805	1.930	1.954	9.183
		5/28	7.895	1.550	1.584	7.900
		5/30	8.560	1.772	1.771	8.800
		6/5	8.490	1.678	1.695	8.365
		6/7	8.992	1.902	1.890	8.785
		6/13	8.995	1.815	1.842	8.885
		6/15	9.070	1.954	1.954	9.183

---

* First egg produced after the larger dose of quinine.

† Dates apply only to the eggs whose weights are given in the adjoining column.

that the beginning of the heavier dosage was followed by a further distinct decrease of egg size and of yolk size. It is thought unnecessary to present the corresponding data for the remaining seven birds since these data for all of the eleven birds are essentially similar.

The last two columns of tables 3 and 4 have been added in order to demonstrate that the yolks produced during dosage with quinine were not provided with the usual or normal amounts of egg albumen. The following explanation is necessary to an examination of these data. Normally, there is of course, a high correlation between the size of the yolk and the total size of the egg. Usually, though not always, the largest eggs contain the largest yolks. The reason for this rule lies in the fact that the stimulus to albumen secretion is a contact stimulus and only that portion of the gland (oviduct) is active which is in contact with the passing object. The time of passage is the same for a small as for a large object.[3] The smaller object therefore secures a smaller quantity of egg albumen. Nevertheless, two different birds do not always supply equivalent amounts to yolks of the same size; nor does the same bird, irrespective of season, age and other conditions, provide uniform quantities of albumen for yolks of equivalent size. In order to make comparisons of the amount secreted under quinine with the normal, the best that can be done is to utilize other eggs laid by the same bird.[4] In tables 3 and 4, opposite the weight of each "treated" yolk, we have therefore placed the weight of that "normal" yolk which, of all the yolks previously laid by the bird, is most nearly equal in size. The total egg weights of the two eggs which bore these two yolks can then be compared in appropriate columns of the tables.

As was stated above, the tabulated data indicate that the yolks produced under quinine did not obtain the normal quantity of egg abumen. Whether there was an actual reduction of the nitrogen present is not definitely shown by our data since it is conceivable, even though not at all probable, that the reduction occurred only or chiefly in the amount of water present in the albumen. Such an alternative assumption is made still less probable by the data, next to be mentioned, obtained by calorimetry of the yolks of the quinine series. The energy values obtained from those yolks supply evidence that the character of the yolks was left unchanged. It is hoped, however, that direct determinations can later be made of the relative amounts of nitrogen in the normal albumen and in that produced under quinine.

---

[3] These facts apply also to the shell-secreting portion of the oviduct.

[4] In tables 1 and 2 the average egg size and yolk size of the eggs laid immediately preceding the beginning of dosage were used for a similar purpose.

Twelve yolks obtained from three of the quinine-fed doves were burned in the bomb calorimeter. When the values obtained from these eggs (table 5) are compared with those obtained from yolks produced by similar untreated females at a corresponding time of the year it is found that the heat values are unchanged from the normal. This is good evidence that the relation of proteins to lipoids[5] in the yolk from quininized birds was unchanged.

TABLE 5

Bomb calorimeter determinations of the energy stored in egg-yolks from females actively dosed with quinine sulfate

NUMBER OF FEMALE	CLUTCH	DATE OF LAYING	TOTAL CALORIES	CALORIES PER GRAM MOIST WEIGHT	CALORIES PER GRAM MOIST WEIGHT IN EGGS OF COMPARABLE FEMALES NOT DOSED*	
A174	B1	3/19/18	6,540	*3,475*	3,462	3,447
	B2	3/21/18	6,225	*3,494*	3,487	3,452
	C1	3/31/18	6,156	*3,414*	3,516	3,373
	C2	4/2/18	6,615	*3,482*	3,485	3,422
A347	C1	3/22/18	5,100	*3,366*	3,362	3,388
	C2	3/24/18	6,321	*3,384*	3,411	3,392
	D1	3/31/18	4,710	*3,329*	3,262	3,348
	D2	4/2/18	5,964	*3,463*	3,344	3,392
152	C1	3/21/18	5,661	*3,543*	3,357	3,406
	C2	3/23/18	6,393	*3,548*	3,542	3,414
	D1	4/1/18	5,565	*3,527*	3,479	3,416
	D2	4/3/18	6,333	*3,580*	3,721	3,404

* Birds of similar origin and eggs taken from as nearly as possible similar dates. The two columns under this heading bear values derived from yolks of different females.

DISCUSSION

One situation presented by these data requires additional comment. It has been shown that after discontinuance of dosage with quinine the production of egg albumen quickly rose to and above normal, while yolk production, after ten weeks, was still below normal. The facts next to be mentioned would seem to make clear the reason for this result.

---

[5] The energy values of yolk proteins and lipoids have been shown by one of us (Riddle, unpublished work) to bear to each other the ratio 5497 : 9020.

Like most tissues of the body the oviduct, flushed freely with an abundant secretion of egg albumen at the time of passage of every egg, rids itself within a few days of any trace of quinine remaining after dosage. The reverse is true for the ovum. It is practically certain that quinine is absorbed by the yolk. Quinine is not oxidized or is oxidized only to a very limited extent in the body and it is probable that the ovum has even less power than other tissues to oxidize it. The long series of smaller slow-growing ova, described in No. I of this series of papers (5), therefore absorb and continue to hold quinine within them; and this absorption and storage must be greatest for the largest of these ova and progressively less for the smaller members of the series.

Bearing in mind that the ova of the series just mentioned are libereted from the ovary in the order of their size it is clear that the most heavily dosed of these eggs were laid earliest after dosage and that the yolks with progressively less absorbed quinine form the series of yolks of progressively increasing size as found in the post-treatment period. These facts also supply the reason for the first clutch of eggs laid after dosage containing, in nine of the ten cases, a smaller yolk than was found in any egg from the dosing period; for, this pair of eggs had been dosed for a longer period than had the preceding pair and some quinine was probably still to be obtained from the blood while this pair (but not the still later pairs) was undergoing the short final period (four to five days) of rapid growth.

It thus seems probable that the presence of quinine accounts consistently for the observed fluctuations in egg albumen production and for yolk size. If—as partly noted in the introductory of this paper—earlier studies on the effects of quinine are correctly interpreted as effects on the nitrogen metabolism rather than on oxidation in general, the reduction of yolk size in the present series of ring-doves would then seem to have been accomplished not by a raising or lowering of the general metabolism but by modification of only a part of it. Probably modifications thus limited in their scope do not provide much hope of being perpetuated in the developmental capacities of the organisms which may arise from these germs; and such modifications are apparently not of the sort which might be expected, from the results of our earlier studies, to effect a modification of sex.

The observed effects of quinine on the activity of the oviduct are fully consonant with previous observations on the nitrogen conserving action of the drug. We are inclined to interpret the effects observed in the ovum (yolk) in essentially the same way. The presence of quinine places a check upon the usual or characteristic transformation of the nitrogenous compounds in the ova. The actual size attained by the latter is, in these cases, probably a resultant of restrictions placed upon the utilization of nitrogenous substances only.

CONCLUSIONS

When small doses of quinine sulfate are fed to laying ring-doves the yolk size and total size of the eggs produced are much decreased.

The reduction of total size is partially to be accounted for by the reduced size of the included yolks. There is, however, a clearly demonstrated additional and abnormal decrease in the amount of egg albumen produced under quinine.

These effects of quinine on yolk size are continued for at least several weeks after the dosage is discontinued. In the course of a few days or weeks, however, the eggs from treated birds contain abnormally large amounts of albumen. Ten weeks after dosage the yolks (ova) approximate but do not attain their normal size.

The relative proportion of protein to lipoid which enters into the composition of the smaller yolks produced under quinine is probably unchanged.

The known tendency of quinine to reduce the destruction of the nitrogenous components of the tissues, as measured by the recovery of nitrogen in the urine, is found to apply to the secretory activity of a gland (oviduct) whose product is entirely of protein nature.

It seems probable that the observed effects of quinine on the ova (yolks) should be similarly interpreted. The reductions and fluctuations in size of the ova are consonant with the view that, in these cases, the size attained is governed by restrictions placed upon the protein metabolism rather than upon the general metabolism.

BIBLIOGRAPHY

(1) v. Noorden and Zunz: Arch. f. Anat. u. Physiol, 1894.
(2) Hertwig and Hertwig: Jena Zeitschr. f. Med. u. Naturwiss., xx, 120 and 477.
(3) Binz: Arch. f. mikr. Anat., iii. Virchow's Arch., xlvi and li. Exper. Untersuch. ueber das Wesen der Chininwirkung, Berlin, 1868. Arch. f. Exper. Path. u. Pharm., i, v, vii.
(4) Riddle: Sci., N. S., xxxv. Abstract, March 12, 1912, 462. Bull. Amer. Acad. Med., 1914, xv. Amer. Nat., 1916, 1. Later papers.
(5) Riddle: This Journal, 1916, xli, 387.

POST-MORTEM MELANIN PIGMENT FORMATION IN PIGMENTLESS RETINAS AND CHOROIDS OF WHITE RING-DOVES

OSCAR RIDDLE AND VICTOR K. LA MER

From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, Long Island, N. Y.

Received for publication July 13, 1918

INTRODUCTORY

The results described in this paper are a contribution to the following subjects: *a*, The mechanism of melanogenesis; *b*, the conditions necessary for post-mortem pigment formation; and *c*, the rôle of the hypothetical "genetic factors" in the inheritance of color.

These studies are based on the embryonic tissues of white ring-doves or of later generations of "extracted" whites from crosses with blond ring-doves. These birds have pigmented irides and non-pigmented retinas and choroids and their pigmentless choroid, skin and (approximately pigmentless) feathers separate them (*Streptopelia alba*) genetically from the next least pigmented species (*St. risoria*) of the genus. The following brief statement of the new facts obtained on the three above-mentioned subjects may be made in advance of a detailed consideration of the data:

a. A tissue which in normal living conditions can form no melanin pigment is shown to be capable of melanin formation under other conditions; the present known means of the attainment of these conditions involve the death of the tissue and a considerable post-mortem period. A certain grade or degree of tissue differentiation must have been attained before post-mortem melanin formation can occur. In the embryonic tissues studied by us the retina must have proceeded to a full 3-day stage[1] before such pigment can be made to form. It is of interest therefore that in the nearest related species—the blond ring-dove (*St. risoria*) —the pigment begins to form at this same time. This embryonic tissue retains its power to form pigment post-mortem from the 3-day stage to at least the 12-day stage of development. At the hatching stage (15-day embryo) the pigment apparently does not form at moderate temperatures and without excess of oxygen. Higher temperatures with excess of oxygen have not been tried on these later stages.

---

[1] The 3-day dove embryo is approximately equal in development to a 4-day chick embryo.

b. Free oxygen is necessary for a post-mortem formation of melanin pigment. This pigment does not form in an atmosphere of CO_2. The pigment will form in a tissue killed in $HgCl_2$. It has failed to form in tissues first subjected to a temperature of 100°C.

c. Current theories of the inheritance of color, assume that these white animals—almost complete albinos—and white hybrids generally fail to produce color because discreet, segregable, germinal bases, called "color factors," fail to gain admittance into the germs which gave rise to them. Under this hypothesis the retina and choroid of these white ring-doves are and must be pigmentless because some of the necessary segregable germinal units are "absent" in the developed tissues. A modification of this hypothesis permits the assumption that the necessary factors may be present but are inactivated by the presence of other segregable germinal units designated "inhibitors." The present results demonstrate that under changed environmental conditions or under another grade or level of conditions, the tissue bears all that is necessary for pigment formation. One needs to supply no new hereditary unit nor extirpate an inhibitor to obtain an abundant supply of melanin pigment. The differences involved reveal themselves therefore in quantitative rather than in qualitative terms. The results thus offer confirmation of the view of melanin pigment formation and inheritance which was advanced by Riddle (1) in 1909.

CONSIDERATION OF PREVIOUS WORK

Bourquelot (2) and Bertrand (3) found that in dead or dying mushrooms there arises a dark color which is due to the action of an oxidase upon an aromatic substance contained in the tissue. This was the first established important fact concerning post-mortem pigment formation; at the same time it has supplied the basis for pratically all later work on the mechanism of pigment formation in living animals and plants. Nearly all of the later work has dealt with pigment formation in the living organism or (chemical studies) *in vitro*. So far as known to us, only the few studies made in Meirowsky's laboratory have considered the formation of melanin pigment in animal tissues after death. Those studies, however, furnish important information on the subject. The first of these publications appeared in 1908. For the purposes of the present paper only the following brief statement concerning the results obtained in this earlier work need be made.

Meirowsky (4) first demonstrated that melanin pigment is formed after death *a*, in human skin from various portions of the body and in scar tissue; *b*, in the embryonic epidermis of the cat, pig and dog.[2] Concerning the mechanism of post-mortem pigment formation Meirowsky showed *a*, that the first appearance of such pigment requires a considerable period of time;[3] *b*, at 56°C. the pigment was produced in human skin in about one-half the time required for its production when kept at 36°C.; *c*, the pigment was formed in pieces of skin that had been subjected to "boiling" in water. Meirowsky favored the view that the melanin production is brought about by enzyme action.

The above studies were continued by Königstein (5) who more carefully studied the conditions under which post-mortem pigment formation occurs. It was found *a*, that pieces of skin immersed in physiological salt solution do not "blacken" at a temperature of 37°C.; *b*, pieces of boiled skin and skin fixed in 1 per cent sublimate or 10 per cent formalin, when dried in Petri dishes and placed for "several hours" at 56°C., become "coal black;" *c*, the "drier the preparation and the higher the temperature, the greater is the degree of pigmentation." Königstein speaks against a pure "ferment" theory being able to account for the results and cites the probable destruction of the enzymes by boiling.

Two further results of Königstein's study will be specially noted and a word of discussion added. *d*, It was found that pieces of skin boiled in alcohol or ether would form the pigment but pieces boiled in acetone would not (except when later heated in soda solution). Since alcohol and ether dissolve lipoids and acetone precipitates them, Königstein inclines to the conclusion that the lipoids stand in a close relation to the formation of the pigment. We may remark that this is not a necessary inference from the data and that the facts now known concerning the chromogens necessary for melanin formation attest the much greater importance of certain constituents of protein. *e*, Finally, the rôle of oxidation and reduction was partially investigated with essentially negative results. In view of the results of the present writers the following statement is of interest:

---

[2] The title of Meirowsky's paper indicates observations on pigment formation in the *eye* as well as the skin, but *post-mortem* pigment formation seems not to have been observed in the eye. His study was, in fact concerned less with the mechanism of pigment formation, post-mortem or otherwise, than with other questions; among these, the origin of pigment independently of melanoblasts (chromatophores), the migration of melanoblasts, the source and mother substance of melanin, etc.

[3] Less time was required in two cases: Skin from a patient dead of Addison's disease and pieces of skin which before death were subjected to Röentgen rays.

> Um zu ermitteln ob ein oxydation prozess die Pigmentbildung begünstigt wurden Hautstüche durch 48 Stunden hindurch in geschlossenen Kolben in je einer CO_2O, or H, Atmosphere ausgesetz ohne dass jedoch in einem der 3 Versuche eine Veränderung zu bemerken war.

Our data demonstrate that, at least for the tissues studied by us, forty-eight hours is not time enough to obtain the pigmentation; all of three days is required. We have demonstrated also that the pigment studied by us can not form at all in CO_2, while in all percentages of oxygen, between the amount present in air and 100 per cent, the pigment forms if a period of three days or slightly longer is given for the test.

In a still further continuation of the above studies Winternitz (6) made the interesting observation that drying the skin produces a dark coloration in it which is removed when the dried colored skin is later soaked in water. The meaning of this observation is not clear to us. This investigator also treated pieces of skin with various oxidizing and reducing agents and concluded that

> reagentien von nicht bedeutender Konzentration hindert das Eintreten des postmortalen Verfärbung nicht.

Further, from the skin of rabbits a substance was obtained which was thought to be chemically similar to adrenalin. This last-named observation is of some interest in connection with a post-mortem formation of a blackish pigment in the adrenal itself as was noted by Meirowsky (7). The latter author concluded, however, that the pigment produced in these adrenals is not melanin because, unlike that formed in the skin, it is not in "granular" form. This objection seems unimportant. In defining "melanins," first consideration may well be given to the chemical nature of the pigment and its mother substance (chromogen) and to the nature of the processes concerned in the transformation of the latter into a colored compound.[4]

---

[4] Abderhalden and Guggenheim (1908) have shown that adrenalin, under the influence of *tyrosinase*, produces dark or dark red flocculi.

PRESENTATION OF DATA

The present studies were undertaken primarily because one of us (Riddle) had occasionally observed in connection with other studies, during two or three previous years, the presence of retinal pigment in some dead embryos of birds (*St. alba*, and some "extracted" whites) known to be incapable of forming such pigment during life. In several of these cases it had been noted that the pigment was found only in a single eye and that this eye was usually or always the one (right) in contact with the air chamber of the egg.[5] It had also been observed that such unexpected pigment was found only in embryos that had died some days before the egg was opened for examination; and further, such eggs seemed often to have especially large air cavities, i.e., these eggs had lost an unusual amount of water and had admitted a correspondingly unusual amount of air. For the purposes of extensive studies being conducted by the senior author, it became necessary to

TABLE 1

Pigment formation from eggs desiccated (over H_2SO_4*) while kept for a period in an incubator (103° F.); later incubation under birds*

PARENTS	EGGS LAID*	DATE TO DESICCATOR; PREVIOUS RATE OF LOSS OF WEIGHT IN MILLIGRAMS PER HOUR	SERIES OF DAILY WEIGHINGS; AND RATE OF LOSS IN MILLIGRAMS PER HOUR					REMOVED FROM DESICCATOR	DATE OF HATCHING OR OF EXAMINATION	PIGMENT IN	
			I	II	III	IV	V			Iris	Retina-choroid
91	4/24	4/26; 5.4	4.9	4.7	5.8	5.2	5.0	5/6, p.m.	Alive 5/14	Traces	None
188a	4/27	4/28	4.4	3.3	3.6	4.0	3.8	5/6, p.m.	Hatched 5/13	Inner ring	None
	4/29	4/29	4.5	4.6	4.8	4.9	5.2	5/9, p.m.	Hatched 5/15	Inner ring	None
2	4/27	4/28; 3.6	4.5	4.0	4.1	3.8	3.7	5/6, p.m.	Partly hatched 5/14	Inner ring	None

* Five eggs were used; one (4/26 of 91) proved infertile.

---

[5] Embryos that have developed more than five or six days lie in such position as to imbed the left eye against the egg yolk and the right eye against the inner shell-membrane limiting the air chamber. This is the position of the right eye in all later embryonic stages in those eggs which have lost very considerable quantities of water. It seemed conceivable that the actual tissues of the right eye might thus be robbed of moisture during or preceding the formation of the pigment.

learn something of the unexpected and unexplained presence of retinal or choroid pigment in dead embryos derived from parents whose offspring should be incapable of producing it. The observations which have been indicated above supplied the reasons for conducting the present observations and experiments along the lines reported here. The known facts of melanogenesis were further reason for an examination of the relation of oxygen (and of carbon dioxide) to the process.

A group of eggs was placed for a period of eight to ten days in an incubator and desiccated at approximately twice the normal rate.[6] These eggs were later placed under birds and hatched (table 1). The eyes of these birds had the normal distribution of pigment, this being present in the iris and wholly absent in the retina and choroid. A considerable number of embryos, of late and full-term stages, were killed previous to the beginning of these experiments in order to supply control data. It seems wholly unnecessary to publish these data since those of table 1 and of all individuals of other tables which survived in the experiments are quite identical with the control.[7] The amount of desiccation obtained in this experiment had therefore no effect upon the pigmentation of embryos which continued to live.

The desiccation of a second group of eggs was carried out, but in connection with incubation in a 100 per cent oxygen atmosphere (table 2). It will be seen that practically all of the embryos died very soon after being placed in the oxygen. A comparison of the ages of the embryos when placed in oxygen, and the estimated ages of the embryos when these were examined several days later, shows conclusively (see columns 5 and 6) that death was caused by the gas[8] into which they were placed. The important fact to be observed in the data of this table is that the three embryos which had attained an age of three to four days or more were the only ones which formed pigment in the eye, and that these three formed pigment not only in the iris where it occurs normally in life, but also in the retina-choroid where it does not form during life. A 2-day embryo failed to form any pigment in either iris or retina and a 3- to 4-day embryo had formed only traces in the normally pigmentless retina. Further, at the time of examination these embryos had been dead for eight days, during three to five of which they had been in 100 per cent oxygen.

---

[6] This rate of loss under normal incubation is considerably higher during the first twelve to twenty-four hours after laying than on subsequent days.

[7] The control data for post-embryonic stages are given in table 10.

[8] The oxygen gas doubtless contained some impurities. Oxygen prepared by the Linde Air Products Company was used in all our experiments.

TABLE 2

Pigment formation from eggs desiccated (over H_2SO_4 *in incubator) for short periods, then continued desiccation and incubation in (nearly) 100 per cent oxygen (fatal dosage) for a few days; still later incubation of some of the dead embryos (first 4 eggs of table) under birds*

PARENTS	EGGS LAID	DATE TO DESICCATOR	RATE OF LOSS DURING 5 DAYS	AGE WHEN O_2 WAS ADDED	AGE OF EMBRYO	DATE OF RECORD	TIME AFTER DEATH	TIME IN O_2	PIGMENT IN	
									Iris	Retina-choroid
				days	*days*		*days*	*days*		
91	5/3	5/3 ; 5:40 p.m.	5.2	7.0	7	5/18	8.0	3.0	Very black	Very black
	5/5	5/5 ; 1:30 p.m.	6.5	5.0	4–4.5	5/18	8.0	3.0	Black	Black
261	5/6	5/7 ; 12:30 p.m.	3.2	4.0	3–4	5/18	8.0	5.0	Black	Traces
	5/8	5/8 ; 10:30 a.m.	7.7	2.5	2	5/18	8.0	4.0	*	*
161d	5/8	5/9 ; 1:30 p.m.	3.2	2.0	0	5/15	Infert.			
	5/10	5/10; 2:30 p.m.		0.5	Broken, p.m.	5/11	Infert.			
188a	5/8	5/8 ; 6:00 p.m.	4.5	2.0	2.5	5/17	7.0	7.0		
	5/10	5/10; 10:15 a.m.	6.3	0.5	1 (?)	5/18	7.0	7.5		
170	5/9	5/9 ; 11:30 a.m.	5.8	1.0	1–2	5/15	5.0	5.0		
	5/11	5/11; 10:30 a.m.	7.8	0.0	1 (?)	5/18	6.0	7.0		
91	5/12	5/12; 6:00 p.m.	6.2	0.0	1 (?)	5/18	5.0	6.0		
	5/14	5/15; 11:30 a.m.	9.8†	1.0	1–1.5	5/18	2.5	3.0		
261	5/15	5/15; 6:00 p.m.	7.2†	1.5	1.5‡	5/19		3.8		
	5/17	5/18; 12:00 m.	6.0†	0.0	1.5–2	5/19	Alive§	2.0		

* The blank spaces indicate no pigment, but in addition they direct attention to the fact that the cause of this is connected with the very immature and undifferentiated state of the embryo. Neither normal nor post-mortem pigment formation has been observed in embryos of less than 3 days of development.

† The period here was for less than 5 days; the rate of loss is usually greater during the first days after laying. The larger figures of this column are usually those for *larger* eggs; the second egg of the pair is larger in most cases.

‡ The embryo (if ever present) had wholly disappeared; this figure is for the stage attained by the *vascular area*.

§ Very little blood in vascular area.

A similar result was obtained on another group of embryos from eggs which were not desiccated (table 3), but otherwise received the same treatment as the above. Desiccation therefore was not a real factor in the cases considered above. It doubtless accompanies a ready ingress of air or of oxygen. Table 3 shows that retinal pigment formed in four of the six embryos which had attained an age of four days or more before death and which in addition had been dead for three or

TABLE 3

Pigment formation from eggs incubated but not desiccated for a short period before being placed temporarily in a (nearly) 100 per cent oxygen atmosphere (no method used for the absorption of CO_2, and no desiccation); later incubation under birds

PARENTS	EGGS LAID	DATE TO 100 PER CENT O_2	NUMBER HOURS IN 100 PER CENT O_2	AGE WHEN O_2 WAS ADDED	AGE OF EMBRYO	DATE EXAMINED	TIME FOR POST-MORTEM PIGMENT FORMATION	PIGMENT IN	
								Iris	Retina-choroid
			.	*days*	*days*		*days*		
116m	5/17	5/25; 9:30 p.m.	21.5	8.0	8–9	5/30	4	R., very black L., traces	Very black Traces
	5/19	5/25; 9:30 p.m.	21.5	6.5	6.5	5/30	4	R., (?) L., traces	(?) Traces
170	5/19	5/24; 5:00 p.m.	50.0	5.0	5.0	5/29	4	R., traces L., more	None
	5/21	5/24; 5:00 p.m.	50.0	3.5	4–4.5	5/29	3–4	None	None
188a	5/19	5/24; 5:00 p.m.	50.0	5.0	5.5–6	5/30	5	Black	Black
	5/21	5/24; 5:00 p.m.	50.0	3.5	5–5.5	5/29	2–3	None	None
91	5/21	5/25; 5:30 p.m.	26.0	4.0	4.0	5/30	4	Black	Black
	5/23	5/25; 5:30 p.m.	26.0	2.5	2.5	5/30	4		
117a	5/23	5/25; 5:30 p.m.	26.0	2.0	3.5	5/26	Alive*	(?)	None

---

* This embryo was probably alive when egg was opened May 26 at 7:30 p.m. This error resulted because of the very *few and indefinite blood vessels* present; when candled it seemed infertile. This case and a number of similar instances met with later possibly indicate that the regulatory mechanisms of the early developing embryo include the power to restrict the number of extra-embryonic blood vessels (and the quantity of blood?) when exposed to a highly oxygenated atmosphere. On the other hand, these changes may be due to "poisonous" effects of the gases used.

TABLE 4

Pigment formation in embryos temporarily subjected to abnormally high (oven, 50° to 57°C.) and low (ice box, about 6°C.) temperatures. It was erroneously supposed that the temperature used would kill all embryos within the time limits

PARENTS	EGGS LAID	NUMBER OF HOURS EXPOSED	KILLED OR SURVIVED	AGE WHEN EXPOSED	AGE ATTAINED BY EMBRYO	DATE EXAMINED	TIME POST-MORTEM	PIGMENT IN	
								Iris	Retina-choroid
					(Ice box, about 6°C.)				
				days	*days*		*days*		
261	5/24	19.0	Killed	11.0	11.0	6/15	11	R., traces* L., none	Traces* None
	5/26	19.0	Killed	9.0	9.0	6/15	11	R., dark* L., traces	Dark* (?)
188a	5/28	19.0	Killed	6.5	6.0	6/15	12	None†	None†
	5/30	19.0	Survived	5.0	13.0 (? alive)	6/15	0	Trace†	None†
2	5/28	19.0	Killed	6.5	7.0	6/15	5	R., inner ring L., traces	None
	5/30		Survived (?)	5.0	13.0	6/15	3	Inner ring	None
161	5/29	19.0	Survived	5.5	17.0 (full-term + 2 days)		0	Inner ring	None
	5/31	19.0	Survived	4.0	hatched		0	Dark on 7/18	None
161d	5/30	19.0	Killed	5.0	4.5	6/15	11	R., dark L., none	(?) None
					Oven, 50° to 57°C.				
188a	6/5	19.5	Killed	10.5	10.0	6/21	6	Dark	Black
	6/7	19.5	Killed	9.0	9.0	6/21	5	R., dark L., none	Traces None
116m	6/5	19.5	Killed	10.5	10.0	6/21	6	R., inner ring L., traces	Dark None
	6/7	19.5	Killed	9.0	9.0	6/21	5	R., dark L., none	Dark None
117a	6/6	19.5	Killed	9.5	9.0	6/21	6	Dark	Dark
	6/8	19.5	Killed	8.0	8.0	6/21	5	R., dark L., traces	Dark None

* From 6/10 to 6/15 this egg was kept in a refrigerator; probably little or no pigment could form while kept at this low temperature.

† From 6/12 to 6/15 this egg was kept in a refrigerator.

TABLE 4—*Continued*

PARENTS	EGGS LAID	NUMBER OF HOURS EXPOSED	KILLED OR SURVIVED	AGE WHEN EXPOSED	AGE ATTAINED BY EMBRYO	DATE EXAMINED	TIME POST-MORTEM	PIGMENT IN	
								Iris	Retina-choroid
			Oven, 50° to 57°C. —*Continued*						
				days	*days*		*days*		
91	6 9	19 5	Killed	6 5	6 5	6 21	5	R., black L., black	Black Traces
	6 11	19 5	Killed	5 0	5 0	6 21	5	R., dark L., traces	Dark Traces
2	6 9	19 5	Survived	6 5	11 5	6 21	0	Inner ring	None
	6 11	19 5	Survived	5 0	9 5	6 21	0	Inner ring	None

more days. The two exceptions (young of 170) were embryos of five days or less. An embryo of two and a half days, dead for four days, and a 3.5-day live embryo had formed no retinal pigment.

Two groups of embryos were next killed, and otherwise not treated, in order to learn whether the pigment would form without excess (not more than in air) of oxygen and without desiccation (table 4). One group was killed by low temperature (6°), the other by high temperature (50° to 57°). It will be noted that a few embryos survived these rather extreme temperatures in which they were kept for nineteen hours. Of the thirteen birds of these two groups which attained an age of more than three days, and also remained dead more than three days before examination, ten formed retinal pigment; one was questionable and two failed to form such pigment. The embryos that failed were of six and a half days. None of the five embryos which remained alive showed any trace of retinal pigment.

A group of embryos was next placed in a 50 per cent oxygen atmosphere. The data of table 5 indicates that nine of the ten embryos treated were killed by this degree of concentration of the gas. That all of the embryos had continued to live in the gas for a period of one to two days is shown, however, by an examination of columns 6 and 7 of the table. The three embryos of more than eight days of development all produced retinal pigment; two of six to six and one-half days and one of three to three and one-half days failed to do so. All had four to five days post-mortem as the period during which the pigment might form. Three embryos of three days of development or less formed no pigment.

Another group of quite young embryos, of one and one-half to three days, were placed during four to eight days in 35 per cent oxygen; this percentage, however, at one time became less than normal and killed six of the eight embryos. The embryos ranged from three to ten days of development; the time post-mortem for pigment formation was only one day (two days in one case) and no retinal pigment was

TABLE 5

Pigment formation in embryos from eggs normally incubated for a period and then permanently transferred to (nearly) 50 per cent oxygen in incubator (no desiccation)

Parents	Eggs laid	Date (12:00 m.) placed in O_2	Time of exposure to O_2	Killed or survived	Age when put in O_2	Age reached by embryo; all eggs examined June 29, p.m.*	Number of days post-mortem	Pigment in	
								Iris	Retina-choroid
			days						
188a	6/13	6/23	6	Killed (?)	10.0	12	4.0	R., none (?) L., none	Black None
	6/15	6/23	6	Killed (?)	8.0	10	4.0	R., none L., none	Traces None
261	6/16	6/23	6	Killed (?)	7.0	8	5.0	R., black L., traces	Black Traces
	6/18	6/23	6	Killed (?)	5.0	6–6.5	4.5	R., trace L., none	None None
91	6/18	6/23	6	Killed (?)	5.0	6–6.5	4.5	R., traces L., none	None
	6/20	6/23	6	Killed (?)	3.0	3–3.5	5.5	None	None
170	6/21	6/23	6	Killed (?)	2.0	3	5.0		
	6/23	6/23	6	Killed	0.1	1	5.0		
188a	6/22	6/23	6	Killed	1.0	2–2.5	4.5		
	6/24	6/26†	3	Survived	2.5	5–5.5	0.0	None	None

* On the last day of incubation, June 28–29, the temperature of the incubator got too high—about 109°F.; later examination of the embryos of this series showed, however, that *all* had died before this period.

† This egg in O_2 at 5:00 p.m.

formed in any case (table 6). A very striking fact concerning this series is that there was also *no pigment in the irides of any of this group when they were examined.* This pigment had certainly been present earlier in the irides of the (seven) embryos which were of more than four days of development. Since all suffered a *lack* of oxygen at the time of death and for some time thereafter it is reasonable to ask whether this lack of oxygen caused an actual disappearance of pigment already formed. That this was almost certainly the case is shown by the data of table 9.

TABLE 6

Eggs normally incubated for a short period and then permanently transferred to (nearly) 35 per cent oxygen in incubator. On July 6 and 7 there was a deficiency of oxygen.—less than in the air—which caused the death of 6 of the 8 embryos*

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE OF EXAMINATION	NUMBER OF DAYS POST-MORTEM	PIGMENT IN	
									Iris	Retina-choroid
			days							
161c	6/26	6/29, p.m.	8	Killed (?)*	3.0	9–10	7/7	1	None	None
	6/28	6/29, p.m.	8	Killed (?)	1.5	8.0	7/7	1	None	None
116m	6/27	6/29, p.m.	8	Killed (?)	2.0	8–9	7/7	1	None	None
	6/29	6/29, p.m.	8	Killed (?)	0.4	6.5–7	7/7	1	None	None
91	6/27	6/29, p.m.	8	Survived	2.0	9	7/7	0	None	None
2	6/30	7/3, p.m.	4	Killed	3.0	4–5	7/7	2	None	None
188a	6/30	7/3, p.m.	4	Killed (?)	3.0	5–6	7/7	1	None	None
	7/2	7/3, p.m.	4	Killed (?)	1.3	3–4	7/7	1	None	None

* This was the *initial* percentage of O_2; it was of course continually diminished through the respiration of the several embryos. At the end of the first three days the oxygen chamber supported a flame better than the air and was then refilled. On July 7, however, at the end of a four-day period (and with larger embryos) there was a decided *deficiency* of O_2 in the chamber. On July 3 concentrated NaOH solution was placed in the bottom of the chamber to absorb the output of CO_2; hitherto the CO_2 had been permitted to accumulate. When the chamber was opened on July 7 it showed a partial vacuum, due to the absorption of CO_2

Table 7 supplies details for another group of embryos kept in 35 per cent oxygen. Four of these birds hatched in the oxygen chamber;

TABLE 7

Eggs normally incubated for a period and then permanently transferred to (nearly) 35 per cent oxygen in an incubator—the O_2 supply being automatically maintained at 35 per cent

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE OF EXAMINATION	NUMBER OF DAYS POST-MORTEM	PIGMENT IN	
									Iris	Retina-choroid
			days							
161	7/2	7/11,p.m.*	7	Hatched	9.0	Hatched	7/18	0	Inner ring	None
	7/4	7/11,p.m.	7	Hatched	7.0	Hatched	7/18	0	Inner ring	None
91	7/6	7/11,p.m.	11	Hatched	5.0	Hatched	7/22	0	Inner ring	None
116m	7/7	7/11,p.m.	8	Killed (?)	4.0	10.5–11	7/19	1.0	None	None
161d	7/8	7/11,p.m.	12	Died. Hatched	3	Hatched	7/23	0	Diffuse	None
125y	7/9	7/11,p.m.	8	Killed	2	7–8	7/19	2	None	None
188a	7/9	7/11,p.m.	12	Killed	2	8–9	7/23	3	R., traces L., none	Dark traces
	7/11	7/11,p.m.	8	Dead (?)	0	8–9	7/19	1	None	None
117a	7/10	7/11,p.m.*	12	Killed	1	7	7/23	6	Traces (?)†	Dark
	7/12	7/12,p.m.	7	Killed	0.3	5	7/19	2	None	None
161c	7/10	7/12,p.m.	3	Killed	2	3	7/15	2	None	None
	7/12	7/12,p.m.	11	Killed	0.3	6–6.5	7/23	4.5	(?)	(?)
116m	7/13	7/19,p.m.	6	Killed‡	6	9	7/25	3	R., dark L., none	Dark
	7/15	7/19,p.m.	6	Killed	4	7	7/25	3	(?)	Traces
262	7/15	7/19,p.m.	6	Killed	4	5–6	7/25	4	Traces	(?)†
125y	7/17	7/19,p.m.	6	Killed	2	4–4.5	7/25	3.5	None	None
	7/19	7/19,p.m.	6	Killed	0.3	3.5	7/25	2.5	None	None
91	7/18	7/19,p.m.	6	Killed	1	4–4.5	7/25	1.5	None	None

---

* The eggs of this table which were in the oxygen chamber, 7/11 m. to 7/12 p.m. were in approximately 40 per cent oxygen.

† This embryo was considerably macerated and its tissues fell apart before the examination could be completed.

‡ On 7/24 the automatic oxygen supply did not work properly and there was no excess of O_2 in the chamber. The six embryos then in the chamber were all dead at that time as is shown by the figures for the ages attained by them.

they had pigment in the iris and none in the retina. Eight older embryos[9] had three or more days for post-mortem pigment formation; five of them formed retinal pigment, one failed and two were not successfully examined. Seven had less than three days post-mortem for pigment formation and none formed the pigment; but these, like the case noted immediately above, also *failed to show pigmented irides* and this raises again the question whether a temporary *deficiency* of oxygen may not have caused the disappearance of pigment already formed. For four of these embryos[9] (last two rows or table) we had reason to suspect a deficiency of oxygen soon after their death. It is possible that an unobserved deficiency occurred also earlier; if so this may account for the five similar cases to be seen in the entire table. All of the nineteen embryos of this series attained the age of three or more days before death.

A further series of ten embryos was very carefully, and we believe successfully, kept during ten to twelve days in a 40 per cent oxygen atmosphere. One bird hatched and another partly hatched in the oxygen chamber. All lived very much longer than when placed in higher percentages of oxygen and possibly all would have survived except for a single but considerable aberration of temperature in the incubator (table 8). All except one (two and a half days) of these birds attained an age of eight to fifteen days. Autopsies were made near to what we now recognize as the minimum time, post-mortem, which is necessary for pigment formation. This period for one embryo was four days; it formed a heavy black retinal pigment. For three embryos the period was three days; one formed the pigment and two failed. For three other embryos the period was two days and none formed the pigment. For one bird the period was one day and no pigment was formed. Finally, for two this post-mortem period was less than one day and no pigment was formed.

Tables 3 to 8 show that it was possible to differentiate in the two eyes the amounts of retinal pigment formed post-mortem in fourteen cases. In every case the right eye (which, as earlier noted, is the eye in close contact with the oxygen of the air chamber of the egg) had more pigment. The amount of pigment borne in the irides was differentiated in eighteen embryos; in seventeen of these the right iris had more pigment and in one case it had less. In the exceptional case (table 3) it

---

[9] The first egg of table 8 is included in this statement.

TABLE 8

Eggs normally incubated for a period and then permanently transferred to (nearly) 40 per cent oxygen in incubator—the O_2 supply being automatically maintained at 40 per cent

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE EXAMINED	NUMBER OF DAYS POST-MORTEM	PIGMENT IN Iris	PIGMENT IN Retina-choroid
			days							
161	7/18	7/18,p.m.	6	Killed*	0	3.0	7/24	3	None*	None
	7/20	7/25,p.m.	10	Killed(?)†	5	13.0	8/4	2	Inner ring	None
117a	7/19	7/25,p.m.	10	Killed (?)	6	13.0	8/4	3	Diffuse	None
	7/21	7/25,p.m.	10	Killed (?)	4	13–14	8/4	2	Diffuse	None
91	7/20	7/25,p.m.	10	Hatched	5	Hatched	8/4	0	Inner ring+	None
188a	7/20	7/25,p.m.	10	Killed(?)‡	5	15.0	8/4	0	Inner ring	None
	7/22	7/25,p.m.	3	Killed (?)	3	2.5	7/28	3	None	None
161d	7/20	7/25,p.m.	10	Killed (?)	5	12–13	8/4	2	Diffuse	None
	7/22	7/25,p.m.	10	Killed (?)	3	11–12	8/4	1	Diffuse	None
116m	7/25	7/28,p.m.	12	Killed	3	9.0	8/9	3	R., very black L., dark	Very black None
	7/27	7/28,p.m.	12	Killed	1	7–8	8/9	4	R., very black L., dark	Very black None

* This egg was treated with others recorded in table 7; 35 per cent of O_2 was used for that series.

† On the night of 7/28–29 the temperature of the oven reached 109°F. Although none of the embryos died at that time it is possible that the birds here marked "killed (?)," were at least weakened by this high temperature.

‡ This bird pricked the shell but died before complete hatching.

was not recorded whether the position of the embryo in the egg was normal.

Another fragment of data bearing on this topic is supplied by table 9. In this case twenty-four embryos were killed by a deficiency of oxygen (or poisoning with 100 per cent CO_2). In two cases the amounts of iridial pigment in the two eyes could be distinguished. Here one right iris showed more pigment and one showed less. It will be noted in that group that only four of twenty-four pairs of irides showed any pigment although it is practically certain that all of these irides contained pigment at the time of death. When killed by CO_2 and kept in CO_2 for periods of five to eight days not more than six of the forty-eight irides showed any trace of pigment.

The data of table 9 were obtained with the purpose of learning whether post-mortem pigment formation can occur in the absence of oxygen. The data previously obtained had shown that such pigment can form in oxygen percentages ranging between that present in normal air and 100 per cent. Three separate groups of embryos—as indicated by the divisions on the table—were separately used in this study. Having previously learned that the stage reached by the embryo must be three or more days, only embryos from four to nine days of development were used. The table makes clear the fact that in the absence of oxygen not a single case of pigment (choroid-retinal) formation occurred.

Observations on embryos at the hatching stage (fourteen to fifteen days) are not complete. Three groups of these embryos kept in air at 30°, 50° and 58° respectively, failed to show a formation of pigment. Higher percentages of oxygen and higher temperatures have not been tried. These experiments were not precisely parallel to those made with younger embryos since the eggs were partly opened to make sure that the bird was alive; in some cases the mature embryos were removed entirely from the shell. All these embryos dried faster than the earlier embryos although in the lot kept at 58° moisture was continually kept in the bottom of the containing chamber. It is perhaps conceivable that the drier cornea and sclera greatly lessened the oxygen supply to the choroid and retina. It is also conceivable that the later and more complete differentiation of the outer layers of the eye greatly restrict the diffusion of oxygen into the eye.

In view of Meirowsky's record of the formation of the pigment in boiled pieces of skin, the following incomplete results are perhaps of interest. Six eggs containing embryos were subjected for fifteen minutes to a temperature of 100° to 107°. These eggs were then kept over moisture at a temperature of 52° to 55°C. during four and six days. When these embryos were examined they were found surrounded by considerable moisture and no pigment had formed post-mortem in any case. Since the heating of the eggs and the presence of moisture in considerable amounts may have lessened the amount of available

TABLE 9

Eggs normally incubated for a period and then permanently transferred to (nearly) 100 per cent CO_2 in incubator. Three separate sets of eggs used, as indicated by the divisions of the table

PARENTS	EGGS LAID	DATE PLACED IN O_2	TIME OF EXPOSURE TO O_2	KILLED OR SURVIVED	AGE WHEN PUT IN O_2	AGE REACHED BY EMBRYO	DATE EXAMINED	NUMBER OF DAYS POST-MORTEM	PIGMENT IN	
									Iris	Retina-choroid
261	7/31	8/9, p.m.	6	Killed	9	9	8/15	6	None	None
	8/2	8/9, p.m.	8	Killed	7	7	8/17	8	R., inner ring– L., none	None
91	8/1	8/9, p.m.	6	Killed	8	7	8/15	6	None	None
	8/3	8/9, p.m.	8	Killed	6	5–6	8/17	8	None	None
188a	8/1	8/9, p.m.	6	Killed	8	7.5–8	8/15	6	None	None
	8/3	8/9, p.m.	8	Killed	6	6	8/17	8	None	None
161	8/1	8/9, p.m.	6	Killed	8	7–7.5	8/15	6	Trace ? (inner)	None
	8/3	8/9, p.m.	8	Killed	6	6	8/17	8	Trace ? (inner)	None
116m	8/2	8/9, p.m.	6	Killed	7	6.5–7	8/15	6	None	None
	8/4	8/9, p.m.	8	Killed	5	5	8/17	8	None	None
161d	8/4	8/13, p.m.	8	Killed	9	8	8/21	8	None	None
	8/6	8/13, p.m.	8	Killed	7	7	8/21	8	None	None
2	8/4	8/13, p.m.	8	Killed	9	9	8/21	8	R., none L., trace? (inner)	None
	8/6	8/13, p.m.	8	Killed	7	7	8/21	8	None	None
170	8/7	8/13, p.m.	8	Killed	6	5–5.5	8/21	8	None	None
	8/9	8/13, p.m.	8	Killed	4	4	8/21	8	None	None
116m	8/12	8/21, p.m.	5	Killed	9	8–8.5	8/26	5	None	None
	8/14	8/21. p.m.	5	Killed	7	7	8/26	5	None	None
261	8/13	8/21, p.m.	5	Killed	8	7–7.5	8/26	5	None	None
	8/15	8/21, p.m.	5	Killed	6	5.5–6	8/26	5	None	None
161	8/14	8/21, p.m.	5	Killed	7	6	8/26	5	None	None
	8/16	8/21, p.m.	5	Killed	5	4.5–5	8/26	5	None	None
188a	8/15	8/21, p.m.	5	Killed	6	5.5	8/26	5	None	None
	8/17	8/21, p.m.	5	Killed	4	4	8/26	5	None	None

oxygen, this test is unsatisfactory. The experiment was therefore repeated and modified by removing a part of the shell from the air space in order to permit an ingress of air. These embryos, however, became very dry within forty-eight hours. No pigment had formed; but in view of our results on the younger embryos the time was not sufficient.

TABLE 10

Pigment in eyes of control birds killed at various ages

PARENTS (PAIRS)	YOUNG	AGE	PIGMENT IN		PARENTS (PAIRS)	YOUNG	AGE	PIGMENT IN	
			Iris	Retina-choroid				Iris	Retina-choroid
		days					*days*		
170	1	145	Dark	None	91	9	50	Dark	None
	2	100	Dark	None		10	49	Dark	None
	3	98	Dark	None	161	11	44	Dark	None
	4	74	Dark	None		12	42	Dark	None
188a	5	206	Dark	None	261	13	49	Dark	None
	6	91	Dark	None	262*	14	52	Dark	None
	7	80	Dark	None	116m	15	40	Dark	None
	8	70	Dark	None	118a	16	68	Dark	None

* Parents similar to birds of pair 261. All of the young produced by this pair were of white color. One egg from this pair was used in the studies outlined in table 7.

In a last examination of this point three pairs of 6- to 8-day embryos were used. The three 8-day embryos were killed at 103°; the 3- to 6-day embryos were killed by immersion in a saturated solution of $HgCl_2$, care being taken to prevent the air space of the egg from coming in contact with the solution; the uncoagulated membrane would therefore continue to permit the ingress of oxygen into these eggs. The six embryos were all examined at the end of four days. The three killed at 103° showed no retinal pigment; the three embryos killed in $HgCl_2$ all showed heavy formations of retinal pigment. In our studies there has been no trace of pigment formation in any embryo subjected to a temperature of 100° or more for periods of five to fifteen minutes.

Our observations and data indicate that the post-mortem pigment formation begins at the inner (pupillary) edge of the iris, later appears in the outer iris and still later in the entire retina-choroid area. This series has an interesting parallel in the order in which the pigment arises in the normal ontogeny. All of the several earlier stages (less than 3-

day embryo) of these living white embryos have no pigment in the iris. When the pigment first appears it forms an inner ring to the iris which is later extended to the outer iris. In these doves the retina-choroid area remains pigmentless throughout life but in their closest relative, the blond ring-dove (*St. risoria*), the formation of iridial pigment is *followed* by the pigmentation of the retina-choroid area A histological study of the macerating[10] ocular tissues bearing the pigment was attempted in only unfixed and smear preparations. Aside from the precise placement of the pigment its solubilities and appearance in fresh preparations under the microscope show that it is unquestionably melanin ocular pigment.

POST-MORTEM PIGMENT FORMATION AND THE POSTULATION OF THE ABSENCE OF GENETIC COLOR FACTORS IN WHITE AND ALBINO ANIMALS

On this topic only a few statements will be added to those made in the introductory to this paper. Table 11 has been prepared to show the character and origin of the parents, grandparents, etc., of the pairs which supplied the embryos for this study. None of the birds used is capable of producing offspring of a color other than white when mated to any other of the twenty-two birds of the list. None of the offspring of these pairs ever produces retina-choroid pigment. Under the conditions described above all of them can and do form the pigment.

The bearing of this fact on theories of color inheritance which make use of "presence and absence" and "inhibitor" concepts is fairly obvious. Clearly none of these white animals wholly lacks a discreet, segregable, germinal entity, without whose presence the formation of black melanin pigment is impossible. This fact may assist in prescribing limits to the frequent and enormous liberties taken with the "presence and absence" hypothesis. Concerning the wholly hypothetical "inhibitors"—the convenient and ever-present refuge of some geneticists —it may be said that the facts of post-mortem pigment formation seem to suggest one limit beyond which the imagination of their creators

---

[10] One may recall in this connection that tyrosin was early identified among the products of autolysis by both Jacoby and Salkowski. It is possible that the delay in the post-mortem pigment formation is associated with a delay in the liberation of tyrosin or other chromogen.

TABLE 11

Showing the character and origin of the parents of the embryos used in these studies. Every bird was of white color; all had pigmented irides and non-pigmented choroids. Every pair has produced only white offspring

PARENTS (PAIRS)	SEX	DERIVATION AND RELATIONSHIP	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY THE PAIR	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY PARENTS OF THE PAIR	NUMBER OF YOUNG (ALL WHITE) PRODUCED BY GRANDPARENTS OF THE PAIR
170	♂	Brother *St. alba* ($\frac{7}{8}$)--*risoria* ($\frac{1}{8}$)			
	♀	Sister *St. alba* ($\frac{7}{8}$)--*risoria* ($\frac{1}{8}$)	64*	77	18†
188a	♂	Brother (from same parents as above)			
	♀	Sister	12	77	18
125y	♂	Brother (= offspring of pair 170 above)			
	♀	Sister	5	64	77
117a	♂	(= Offspring of pair 170 above)	3	64	77
	♀	(= Sister to pair 170 above)		77	18
91	♂	(= Brother to pair 170 above)		77	18
	♀	Extracted white from *alba-risoria-douraca*‡	21		
161d	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	3	21	
2	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	3	21	
161	♂	Brother (sire = brother to pair 170 above, and dam = sister to ♀ of pair 91 above)	9	64 5	77
	♀	Sister			
261	♂	(= Brother to pair 161 above)	5	64	77
	♀	(= Offspring of pair 91 above)		21	
161c	♂	Brother (= offspring of pair 91 above)			
	♀	Sister	1	21	
116m	♂	Nearly pure *St. alba*		6	26
	♀	Extracted white, or "mutant" (?) from blond parents	13		

---

* None of the embryos or offspring used in these pigment studies is counted in the figures of this column.

† The female grandparent of pair 170 was an extracted white from a crossing of *St. alba* with *St. risoria*.

‡ The parents were ♂ *alba-risoria* and ♀ *alba-douraca-risoria*, of blond and dark color respectively. All of the white young of this and later generations, mated *inter se* or with *St. alba*, have thrown *only* white offspring

may not go: The regnancy of the color "inhibitor" certainly does not extend beyond death.

The facts of post-mortem pigment formation lend support to the following conclusion of one of us (1), as stated (p. 330) in 1909:

> The specific color of an animal then is an index, not of the presence in the germ from which this animal arose of certain chromogens and specific zymogens, and the absence of a wide series of others; but, this specific color means that a *process* with a wide range of possibilities, *because of a particular physiological state and environmental conditions* has struck this particular equilibrium. One and the same organism has within it all that is necessary to move that equilibrium up or down.

SUMMARY

The post-mortem formation of melanin (black) pigment has been shown to occur in the retina-choroids of white dove embryos of three to twelve days of development. The pigment is not produced in earlier stages and is not as readily produced, possibly not at all, in the full-term or just-hatched embryos. Killing the tissues in $HgCl_2$ does not prevent the development of the pigment. No pigment was formed in the few tested embryos which were previously subjected to a temperature of 100° to 107°C.

The presence of free oxygen is necessary for the production of the pigment. It will form in percentages of oxygen varying between that present in air and 100 per cent. The pigment fails absolutely to form in an atmosphere of CO_2; further, it seems probable that the inner ring of iridial pigment which is present in older living embryos entirely disappears when kept after death in the presence of high percentages of CO_2.

The facts of post-mortem melanin formation have a bearing on current theories of color inheritance and development. They coincide with the view advanced by Riddle in 1909, and present difficulties and limitations to the "presence and absence" hypothesis of color development.

BIBLIOGRAPHY

(1) Riddle: Biol. Bull., 1909, xvi, 316.
(2) Bourquelot: Compt. rend. de la Soc. biol., 1896, lviii, no. 5.
(3) Bertrand: Compt. rend., 1896, cxxii and cxxiii.
(4) Meirowsky: Bibliothek Medizin, Monographien, iv, 1908.
(5) Königstein: Münch. med. Wochenschr., 1909, lvi, 2305,
(6) Winternitz: Arch. f. Derm., 1911, cvii, 293.
(7) Meirowsky: Centralbl. Allg. Path., 1910, xxi, 721.

THE CHEMICAL COMPOSITION OF THE BRAIN OF NORMAL AND ATAXIC(?) PIGEONS

MATHILDA L. KOCH AND OSCAR RIDDLE

From the Psychiatric Institute of the New York State Hospital and the Station for Experimental Evolution, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, N. Y.

Received for publication July 13, 1918

Very few data have hitherto been obtained on the chemical composition of the brains of individuals with hereditary mental deficiency or derangement. Fewer still are such data obtained from otherwise healthy, normal individuals whose brains were prepared for analysis at the moment of death. In no case, so far as we are aware, has it been possible to obtain duplicate samples of abnormal brains and to compare them with normals of essentially the same parentage and of similar age. All of these advantages are present in the case of the brains of the pigeons here described.

A limited number of chemical studies of human brains, from individuals, dead of various diseases and acute infections, have been made. Still other experiments have been conducted with the object of influencing the chemical composition of the brain of various animals by complete starvation, by underfeeding or by the feeding of specific or particular diets. It does not seem to us that the literature bearing upon these topics requires special treatment here since our data were obtained from brains showing hereditary derangement and particularly since in this material there is reason to believe that something of the condition found at death was continuously present since the early development of the embryo and is, quite probably, not to be wholly accounted for by subsequent degeneration.

MATERIAL AND METHODS

The functional abnormality associated with the brains studied is recognizable and describable as a lack of control of the voluntary movements. This has been shown in different degrees by different individual birds. Occasionally a bird deficient in motor control in early life becomes normal at a later period and conversely some birds apparently normal in early life may partially lose the control of their voluntary movements in later life. Usually the derangement persists until death.

It is not yet certain by what name this disorder should be called. Even before the present chemical data were obtained, however, there were good reasons for designating the brain as the seat of the disorder. A preliminary description of the origin and inheritance of the disorder has been published by one of us (1); provisionally the disorder has been called ataxia(?).[1] It may here be stated that the more usual manifestations of the disorder are: Nodding of the head or nodding and swaying of the head and neck; unsteady gait; tipping (somersaulting) backwards or forwards; falling on the side; very irregular flight, the bird even flying backwards. The same bird often exhibits two, three or perhaps all of these irregularities. Practically all affected birds are unable and uninclined to sit on a perch, remaining constantly on the ground or on a flat ledge. In the most affected individuals there seem to be no movements whatever of wholly normal coördination; in average cases, however, the disturbances are much increased under excitement, fear or any attempt at increased or more vigorous movement. In a few cases the movements have seemed fairly normal when the bird was at perfect rest. Several of the affected birds have mated and produced young.

The necessary data for age, sex, brain weight, body weight and degree of ataxia are fully given (table 3) for each individual bird and for each group of five birds whose brains were combined into one sample for chemical analysis. The birds were all killed by decapitation and the brain removed immediately, using the following technique: The skull was opened with small bone-forceps so as to expose the convexity of the brain from the fourth ventricle to the olfactory bulbs. An incision was made just posterior to the fourth ventricle, the brain was then lifted up with forceps and the optic nerves cut close to the eyeballs and the olfactory bulbs left attached to the brain. Each brain was quickly weighed and placed in a sufficient quantity of redistilled alcohol to make the final concentration of alcohol about 85 per cent. Five samples were collected in this way. Two of these were controls, one from younger and one from older pigeons; and three samples were formed from brains of birds showing three different degrees of ataxia.

---

[1] Competent neurologists have undertaken a special study of the brains of these abnormal birds; probably the center or centers of the disorder, or further knowledge of the nature of the disorder, will be disclosed by that study.

In the present paper it is not thought necessary to describe in detail the method (2) used in the determination of the several chemical constituents of the brain further than to say that these brains were allowed to stand in the alcohol for two months, after which a hot alcohol extraction was made, followed by an ether extraction; the brain material was then softened with water after grinding to a fine powder in a mortar. A final 85 per cent alcoholic extraction for fifteen hours completed the extraction. By this procedure the lipoids and water-soluble extractives were separated from the protein residue. The lipoids were then separated from the extractives by precipitation with acid (0.5% HCl and $CHCl_3$), and the sulfur and phosphorus determined in each of these three fractions. Cholesterol was determined in the lipoid fraction.

PRESENTATION OF DATA

Tables 1 and 2 give the analytical results obtained in the normal and ataxic brains. Tables 3 and 4 give general information concerning the nature of the various samples as used for analysis. It is well known that the chemical composition of the brain varies greatly with age; the age of each individual and of each group of individuals is completely given. The sex also is indicated in all cases although it is fairly certain that only slight differences, if any, exist in the percentage composition of the brains of the two sexes.

An examination of the figures of table 1 shows that the group of "most ataxic" pigeons had, among the five groups compared, the highest percentage of water, protein and extractive sulfur; and the lowest percentage of lipoids, phosphatides, and cholesterol. The "more ataxic" group has percentage values which are intermediates of those of the "slightly ataxic" and "most ataxic" groups in practically all of the above-mentioned fractions.

Our own study of these data inclines us to the view that the brains of the ataxic pigeons tend to remain—from a chemical standpoint—in a juvenile or infantile condition. The departures of the various chemical constituents of the ataxic brains from the normal are consistent with the differences known to exist between the brain of the young and the adult. Concerning the relation of age to the chemical composition of the human brain W. Koch (3) concluded as follows:

> Analysis of the brain at different ages shows that with the growth of the brain there is a decrease in the amount of moisture, proteins, extractives and ash; and the cerebrins, lipoids and cholesterin increase. Also there is an increase in the

lipoid sulfur and phosphorus and a decrease in the neutral and inorganic sulfur (=extractive sulfur in tables 1 and 2 of the present paper) and extractive phosphorus.

Koch's published figures on the relation of age to chemical composition of the human brain are placed by us in table 1 to facilitate a comparison between the ataxic and normal pigeon brains in the light of the known changes in composition due to age.

TABLE 1

Constituents of pigeons' brains in percentage of solids

GROUP	I SLIGHTLY ATAXIC (♀♀) 166 DAYS	II MORE ATAXIC (♂♀) 133 DAYS	III MOST ATAXIC (♂♀) 158 DAYS	IV NORMAL (♂♀) 183 DAYS	V NORMAL (♂♂) 106 DAYS	HUMAN BRAIN (13) FOR COMPARISON		
						6 weeks	2 years	19 years
Water in per cent	79.52	79.57	80.25	79.83	79.97	88.78	80.47	76.42
Proteins	49.72	50.86	52.10	50.73	49.99	46.6	40.1	37.1
Lipoids	37.15	36.42	34.87	37.08	36.76	33.1*	47.4*	52.0*
Extractives	13.13	12.72	12.90	12.19	13.24	20.3	12.5	10.9
Phosphatides	22.99	22.39	21.91	23.52	22.93	24.2	25.5	27.3
Sulfatides	6.10	(8.08)	6.26	6.33	6.48	1.6		4.9
Cholesterol	7.37	7.08	6.80	7.50	7.01	1.9	8.7	11.7
Distribution of sulfur								
Protein sulfur	0.527	0.496	0.520	0.483	0.439	62†	59.0	62.0
Lipoid sulfur	0.122	(0.161)	0.128	0.126	0.129	6†	17.0	22.0
Extractive sulfur	0.105	0.104	0.118	0.084	0.099	32†	24.0	15.0
Total sulfur	0.754	(0.761)	0.766	0.694	0.668	0.52	0.58	0.48
Distribution of phosphorus								
Protein phosphorus	0.272	0.289	0.268	0.285	0.295	5‡	6.0	5.0
Lipoid phosphorus	0.892	0.869	0.850	0.912	0.889	54‡	67.0	72.0
Extractive phosphorus	0.341	0.328	0.326	0.304	0.345	41‡	27.0	23.0
Total phosphorus	1.503	1.486	1.444	1.502	1.529	1.72	1.48	1.45

* Total lecithins, kephalins, cerebrins and cholesterin.

† Sulfur in per cent of total sulfur.

‡ Phosphorus in per cent of total phosphorus.

It may be noted that the total phosphorus in the ataxic brains is somewhat less than in the normal. This is contrary to expectation from the standpoint of the figures given for the relation of age to phosphorus content in the human brain. It will be seen, however, that in the more complete figures given for the rat's brain (appended to table 2) slightly less phosphorus was found in the 10-day than in the 120-day rat. The slightly lower total phosphorus values found in the ataxics as compared with normals is therefore possibly not valid evidence against the juvenile character of the ataxic brains.

Details of the distribution of sulfur and phosphorus calculated in per cent of total sulfur and total phosphorus are given in table 2. To table 2 are added for comparison the same fractions obtained from albino rats of various ages. These figures (thus calculated) obtained on the rat indicate: *a*, Protein sulfur is present in smallest amounts in the youngest stages. The "more ataxic" group of brains seems to show this smallest proportion of protein sulfur. *b*, Lipoid sulfur is similarly present in smallest amounts in the youngest stages of the rat. The "slightly ataxic" and "most ataxic" groups of pigeons show lower values than either of the two normal groups. *c*, Extractive sulfur is present in greatest amount in the youngest stages of the rat. It is present in greatest amount in the "most ataxic" pigeons. *d*, Protein phosphorus is apparently less in rats of twenty days than in either older or younger stages. The five groups of pigeons brains show no consistency in this figure, either in reference to age or to affected and unaffected brains. *e*, Lipoid phosphorus is lowest in youngest rats. It is lowest in the much younger normal pigeons but plainly lower in the three groups of ataxics than in the older normals. *f*, Extractive phosphorus is found in greatest relative proportion in the youngest stages of the rat. All three groups of "ataxic" pigeons show higher values than do the older group of normals, but values in close agreement with those of the much younger normals.

DISCUSSION

There are several questions which naturally arise in connection with an examination and interpretation of the analytical results obtained on these pigeon brains. We have sought to supply data probably useful as giving answer to some of those questions, in the form of tables.

Tables 3 and 4 show that the body weight and brain weight of the males used were greater than those of the females and that the average age of the males was less by fifteen days. There is little of real immaturity, however, in either group, the youngest birds (table 4) of either sex showing brain weights nearly or quite equal to the average for their

TABLE 2

Distribution of sulfur and phosphorus in percentage of total sulfur and total phosphorus

GROUP	I	II	III	IV	V
Protein sulfur	69.88	65.12	67.93	69.64	65.80
Lipoid sulfur	16.18	(21.22)	16.70	18.24	19.40
Extractive sulfur	13.94	13.66	15.37	12.12	14.80
Total sulfur (in per cent of solids)	0.754	(0.761)	0.766	0.694	0.668
Protein phosphorus	18.09	19.46	18.52	19.00	19.28
Lipoid phosphorus	59.25	58.44	58.88	60.77	58.17
Extractive phosphorus	22.66	22.10	22.60	20.33	22.55
Total phosphorus (in per cent of solids)	1.503	1.486	1.444	1.502	1.529

The same chemical groups in albino rat of different ages for comparison (14)

GROUP	AGE IN DAYS				
	1	10	20	40	120
Protein sulfur*	31.10 30.00	48.60 44.20	57.50 55.30	65.10 62.40	61.20 62.40
Lipoid sulfur	3.20 2.80	6.10 6.70	6.70 7.50	9.20 10.10	12.80 12.50
Extractive sulfur	65.70 67.20	49.20 49.70	35.80 37.20	25.70 27.50	26.00 25.10
Total sulfur (in per cent of solids)	0.96 1.04	0.72 0.83	0.69 0.70	0.58 0.52	0.55 0.57
Protein phosphorus	13.30	13.00 13.90	6.00 5.80	9.90 7.50	7.40 7.30
Lipoid phosphorus	33.20 33.00	33.80 36.10	52.20 53.50	56.10 58.50	65.80 62.30
Extractive phosphorus	53.50 53.60	53.20 50.00	41.80 40.70	34.00 34.00	26.80 30.40
Total phosphorus (in per cent of solids)	1.82 1.92	1.28 1.48	1.66 1.67	1.55 1.50	1.40 1.44

* Duplicate analyses throughout this part of the table.

TABLE 3

Data on the age, sex, brain weight, body weight and degree of ataxia of the five groups of pigeons which supplied samples for analysis

NUMBER OF SAMPLE	DESCRIPTION OF GROUP		SEX AND NUMBER	AGE	BRAIN WEIGHT	BODY WEIGHT
				days	*grams*	*grams*
I	Slightly ataxic	Females (5)........	♀M357	125	1.780	332
			♀M355	129	1.831	309
			♀K143	143	1.605	297
			♀K175	183	1.835	308
			♀K136	249	1.867	325
Average of sample..................				166	1.784	314
II	More ataxic	Females (2)..........	♀K249	69	1.811	292
			♀M315	127	1.813	336
		Average		98	1.812	314
		Males (3)........	♂M3450	124	1.943	340
			♂K181	161	1.978	331
			♂K180	182	1.955	358
		Average		156	1.959	343
Average of sample				133	1 900	331
III	Most ataxic........	Females (3)..........	♀K242	83	1 800	309
			♀K220	158	1.670	303
			♀K182	185	1.736	340
		Average		142	1 735	317
		Males (2)..	♂K166	172	1 842	350
			♂K170	191	1 897	326
		Average		182	1 870	338
Average of sample..				158	1.789	326
IV	Normal (older)	Females (3)	♀K197	181	1 743	362
			♀K124	186	1 782	349
			♀K117	216	1 935	349
		Average		194	1 82	353
		Males (2)	♂K195	158	2 082	367
			♂K163	173	1 855	382
		Average ...		166	1 969	374
Average of sample				183	1.879	362

TABLE 3—*Continued*

NUMBER OF SAMPLE	DESCRIPTION OF GROUP		SEX AND NUMBER	AGE	BRAIN WEIGHT	BODY WEIGHT
				days	*grams*	*grams*
V	Normal (younger)	Males (5)...........	♂K264	69	1.809	324
			♂K277	83	1.900	357
			♂K260	109	1.940	398
			♂M301	123	2.065	342
			♂K222	144	1.895	380
Average of sample				106	1.922	360

* The age of each bird includes the whole of its incubation period.

TABLE 4

Males and females whose brains were analyzed listed separately and in order of increasing age

FEMALES				MALES			
Number and description of birds	Age	Brain weight	Body weight	Number and description of birds	Age	Brain weight	Body weight
	days	*gms.*	*gms.*		*days*	*gms.*	*gms.*
K249 (more ataxic).....	69	1.811	292	K264 (normal)........	69	1.809	324
K242 (most ataxic).....	83	1.800	309	K277 (normal)........	83	1.900	357
M357 (slightly ataxic)..	125	1.780	332	K260 (normal)........	109	1.940	398
M315 (more ataxic).....	127	1.813	336	M301 (normal)........	123	2.065	342
M355 (slightly ataxic)..	129	1.831	309	M3450 (more ataxic)..	124	1.943	340
K143 (slightly ataxic)..	143	1.605	297	K222 (normal)........	144	1.895	380
K220 (most ataxic).....	158	1.670	303	K195 (normal)........	158	2.082	367
K197 (normal)..........	181	1.743	362	K181 (more ataxic)....	161	1.978	331
K175 (slightly ataxic)...	183	1.835	308	K166 (most ataxic)....	172	1.842	350
K182 (most ataxic).....	185	1.736	340	K163 (normal)........	173	1.855	382
K124 (normal)..........	186	1.782	349	K180 (more ataxic)....	182	1.955	358
K117 (normal)..........	216	1.935	349	K170 (most ataxic)....	191	1.897	326
K136 (slightly ataxic)...	249	1.867	325				
Average.............	156	1.785	324	Average.............	141	1.930	355

sex. The additional fact may be stated here that our determination of the body weights of several much older birds of this strain, normals and ataxics, indicates an average body weight scarcely, if any, higher than that shown in the birds which supplied the samples for analysis. It is not yet definitely known whether the brain weight of these birds increases beyond the age represented by the birds used in our analyses;

the fact referred to above, however, concerning the approximate average brain size of the youngest birds selected, would argue against any later considerable increase in brain weight.

A summary comparison of some of our results with those of earlier investigations on the pigeon's brain is given in table 5. Lukjanow (4), who seems to have used a smaller variety or species of pigeon than ours, published no data as to the variety or the age of the birds studied by him. Funk (5) also failed to note the age of the pigeons used in his studies. The brain weights and body weights of his birds indicate that in these respects the birds used were fairly comparable with those used by us.

The questions just raised concerning brain size and body size perhaps deserve a short additional statement. Our ataxic birds were somewhat undersized as compared with the normals although not markedly so; and the fact that the older ataxics were larger than the younger ataxics, essentially in the order of age, makes it reasonably clear that these birds had grown continuously although more slowly than the normals. It is possible that the "most ataxic" birds, because of the extreme awkwardness of movement, sometimes failed to obtain as much food as they wanted. On this point it may be noted that Hatai (6) has shown that temporary underfeeding, in the albino rat, leaves unchanged the relation of brain weight to body weight. Donaldson (7) observed a 2.5 per cent increase in the relative brain weight of rats given special opportunities for voluntary exercise. It is probable that the ataxic pigeons do not exercise as much as the normals and part of the discrepancy in brain weight of these ataxics may be thus explained.

The studies of Mott (8), Carlyll and Mott (9) and S. A. Mann (10), on the brains of children dead of amaurotic dementia (Tay-Sach's disease) are of much interest in the present consideration of the chemical composition of the brain in hereditary mental disease. Attention can here be directed, however, only to some seemingly significant agreements in the two series of data and to the nature of the interpretations offered by the above-mentioned investigators for the observed chemical composition of the brain in amaurotic dementia.

The results of Mott and of Mann[2] indicate higher moisture values in this disease. Notably less lipoid phosphorus and sulfur with a corresponding increase of the extractive forms were found. The values in the affected brains for total extractives, cerebrosides and cholesterol seem unchanged

---

[2] Mann's analyses were carried out by the method of W. Koch.

TABLE 5

A comparison of present (normal and ataxic) with earlier data obtained from investigations on the pigeon's brain

DESCRIPTION	BODY WEIGHT	BRAIN WEIGHT	WATER IN BRAIN	INVESTIGATOR
Normal				
	grams	*grams*	*per cent*	
10♂	292.6	1.908	79.94	Lukjanow
10♀	256.6	1.850	80.37	
Average	274.6	1.879	80.16	
Average	320	1.80	78.62	Funk
IV {3♀ 2♂}	362	1.879	79.83	Koch and Riddle
V 5♂	360	1.922	79.97	
Average	361	1.900	79.90	
Experimental (and abnormal)				
Starved for:	*Init. and fin.*			Lukjanow
154 hours, ♂	299 to 189	1.881	79.73	
151 hours, ♀	274 to 190	1.887	79.82	
Average	287 to 190	1.884	79.78	
Starved till dead ♂	288 to 159		82.44	
Starved till dead ♀	242 to 149		80.79	
Average	265 to 154		81.62	
Polyneuritis	300 to 232	1.75	79.42	Funk
Cured of polyneuritis	314 to 259	1.82	78.50	
Underfed	352 to 269	1.98	78.14	
Average	322 to 253	1.85	78.69	
I. Slightly ataxic 5♀	314	1.784	79.52	Koch and Riddle
II. More ataxic {2♀ 3♂}	331	1.900	79.57	
III. Most ataxic {3♀ 2♂}	338	1.789	80.25	
Average	328	1.824	79.78	

or inconsistently changed. A very pronounced change was found in the relation of nucleo-proteins to simple proteins. The former were much decreased. This result also is paralleled in the pigeon brains, as will be seen from the following calculation[3] of the amount of nucleo-protein in the five groups of pigeon brains: I, 5.94; II, 6.38; III, 5.78; IV, 6.30; V, 6.51. Perhaps the most noticeable difference in the affected human and pigeon brains is that total sulfur and total phosphorus were both found by Mann to be decreased in the human, while in the pigeon we find the total phosphorus decreased and the total sulfur increased. The chemical variations from the normal as observed in the ataxic pigeon brains and in human amaurotic dementia are apparently alike in kind.

The above-mentioned workers did not in general interpret their results in terms of infantilism or under-development. The following quotations from their publications, which are also concerned with clinical and histological studies, are given as indicative of their interpretations and conclusions:

Mott (1905) states that

> The profound affection in the physiological functions of the central nervous system which characterizes this disease may be especially associated with a bio-chemical change in the metabolism of the nucleus. The cause of this regressive metabolic metamorphosis may be an inherent lack of specific energy, racial or familial, of the neurones and possibly some hitherto undiscovered bio-chemical alteration of the blood or lymph. The existence of the neuro-fibrils, which may be regarded as the conductile structure of the neurones, which clinical facts tend to show had ceased to function, would support the view either that the Nissl substance which had disappeared was itself an important agent in neural function, or that it was an antecedent of a substance at the synapses. The increase of neuroglia fibril substance to an abnormal degree in case I, so that the brain weighed heavier than that of a normal adult, accounts for the large amount of simple proteins and the great diminution of nucleo-proteins.

Carlyll and Mott later (1911) examined seven cases of Tay-Sach's disease, two of which were analyzed chemically by Mann. These authors conclude that the cause of this disease is "probably a failure in the germinal determinants of the nervous system peculiar to the Jewish race," and set forth the hypothesis that

> It may be due to a failure in the nuclear material of the neurone to build up the nucleo-protein Nissl substance out of lipoid substances contained in the cyto-

---

[3] Obtained from protein phosphorus by factor (175.4) as worked out by Levene (11).

plasm which first has to be decomposed by nuclear ferments. . . . . The chemical analysis does not throw much light upon the question; the diminution of the lipoid form of phosphorus and sulfur is probably due to the diminution of myelin owing to the failure of development[4] of the myelinated fibers. The corresponding increase of extractive forms of phosphorus and sulfur may be possibly due to a breaking down of the more complex to simpler forms of lipoids.

Mann states that some (not all) of the changes observed "may be explained as the result of nutritive changes."

We believe that the care used in the preparation of the five groups of brains and the degree of accuracy of the methods of analysis justify our conclusions concerning the differences found in the analytical results. We hope, however, to be able further to test and amplify the results reported here by an investigation in which the cerebellum is analyzed apart from the remainder of the encephalon.

The birds used are all inbred for two to four generations. The original ataxic female—which arose under conditions known in pigeons to produce *weakened* germs,—is a common ancestor of them all (normals and ataxics). The stock is mongrel common pigeon but nearly all of the birds are three-fourths homer (*C. tabellaria*) and one-fourth mongrel; a few are about one-fourth rock-pigeon (*C. livia*). Since the derangement (ataxia?) characteristic of these birds arose as a wide variation (mutation?) under conditions known (12) to favor or induce the production of females, individuals of shorter life-term, and less vigor, it is of added interest that the chemical composition of these ataxic brains indicate a retardation or restriction of the development of an important part[5] of the central nervous system.

SUMMARY

The brains of birds which have very little control of the voluntary movements (ataxia?) have been analyzed. The data thus obtained are compared with similar data obtained on the brains of normal birds of the same parentage.

The derangement first appeared (mutation?) under conditions known to lead to weakness in the offspring. The disorder is exhibited in all degrees and has been inherited undiminished to the fifth generation.

---

[4] This statement is, of course, somewhat similar in nature to the interpretation applied generally by the authors of the present paper.

[5] It is, of course, not necessarily to be inferred that the whole of the encephalon is chemically under-differentiated. Possibly some particular localized areas are solely or chiefly involved.

Usually it is shown from the earliest age of the bird and is probably present throughout the whole development of the bird.

The brains of the affected individuals show increased values for moisture, protein and extractive sulfur; decreased values for lipoids, phosphatides and cholesterol. In general the less ataxic individuals show values intermediate to those of the normals and most ataxics. The distribution of sulfur and phosphorus in the various chemical fractions was determined.

The results of the analyses of the affected brains are interpreted as suggesting a chemical under-differentiation or immaturity of these brains. The brains of affected birds of approximately mature age are chemically more like the brain at earlier stages of development.

BIBLIOGRAPHY

(1) Riddle: Proc. Soc. Exper. Med. and Biol., 1918, xv, 56.
(2) Koch: Journ. Amer. Chem. Soc., 1909, xxxi, 1340. (Recently republished with slight modification by Koch and Voegtlin, Carl. Hyg. Lab. Bull. 103, 1916).
(3) Koch: Arch. Neurol. and Psych., 1909, iv, 44.
(4) Lukjanow: Zeitschr. f. physiol. Chemie, 1889, xiii, 339.
(5) Funk: Journ. Physiol., 1912, xliv, 50.
(6) Hatai: This Journal, 1904, xii; Journ. Comp. Neurol. and Psych., 1908, xviii.
(7) Donaldson: Journ. Comp. Neurol., 1911, xxi.
(8) Mott: Arch. Neurol. and Psych., 1905, iii.
(9) Carlyll and Mott: Arch. Neurol. and Psych., 1911, v.
(10) Mann: Ibid. (Appendix to above paper of Carlyll and Mott).
(11) Levene: Arch. Neurol. and Psych., 1899, ii.
(12) Whitman: Posthumous works, ed. by O. Riddle, Carnegie Inst., ii (in press).
Riddle: Amer. Nat., 1916, l and earlier papers cited there.
(13) Koch and Mann: Journ. Physiol. (Proc. Physiol. Soc.), 1907, xxxvi.
(14) Koch and Koch: Journ. Biol. Chem., 1913, xv.

A NEW FORM OF AEROTONOMETER

PAUL M. O'SULLIVAN

From the Department of Physiology, The University of Toronto

Received for publication July 19, 1918

In the series of experiments in which we undertook to investigate the possibilities of the lung as an aerotonometer, we used in the first instance, for purposes of comparison, one of Krogh's aerotonometers (1). Important difficulties were encountered in using this tonometer. First, the time taken to reach equilibrium. Under favorable conditions it takes at least twenty minutes and usually longer owing to the fact that the blood tends to run in streaks down the tube, thus diminishing the surface available for diffusion. Second, only a small quantity of gas is obtainable for analysis and since it is desirable that all analyses be made in duplicate, this is a serious drawback.

The following is a description of a new type of aerotonometer designed by the late Prof. T. G. Brodie, which has been found to be very satisfactory. It consists of two horizontally placed glass cylinders, one inside the other. The outer tube is 236 mm. in length and 27 mm. in outside diameter. At either end the tube is flanged to 30 mm.; the inside diameter is 23.5 mm. The inner tube is supported by a ground glass joint to one extremity of the outside tube. Its length is 223 mm. and outside diameter 18 mm. It is closed at its free end and the other end projects 20 mm. from the outside tube to afford attachment to a wheel which is revolved by belting from the shafting. The outer tube rotates on a ground glass surface, about a rubber cork surrounded by a shell of ground glass. This cork fits into the other flanged end of the outside tube. Through the cork pass three tubes; a fine silver tube, *A*, to bring the blood into the tonometer; a thick glass tube, *V*, which carries the blood back, and another fine silver tube, *X*, from which air samples may be taken. The blood enters through the fine silver tube, situated lowest in the cork. This tube carries the blood to the far end of the apparatus. The blood is returned by the middle thick glass tube which dips down to the bottom of the tube immediately on its entrance. The space between the two cylinders is kept half full of blood so that the upper half is filled with gas. The fine silver tube entering fom the top of the cork is in communication with this gas. On its exit the tube bifurcates, one limb leading to a manometer, M, and from the other limb samples of this gas may be extracted for analysis.

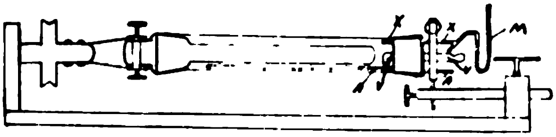


Fig. 1

The volume between the two cylinders is about 34 cc. When half full of blood this leaves 15 cc. of gas, an ample volume for several analyses.

In estimating the relative value of different tonometers, great stress has been laid on the "specific surface" which, as defined by Krogh (2), is the ratio of the volume of gas in cubic centimeters to the surface available for diffusion in square centimeters.

The specific surface of this tonometer is about 7.3, as shown in the following calculation.

$$\text{Specific surface } \frac{\text{Area}}{\text{Volume}} \; \frac{\tfrac{1}{2}(2\pi\gamma l + 2\pi\gamma' l)}{\tfrac{1}{2}(2\pi\gamma^2 l - \pi\gamma'^2 l)} = \frac{2\pi l(\gamma + \gamma')}{\pi l(\gamma + \gamma')(\gamma - \gamma')} =$$

$$\frac{2}{\gamma - \gamma'} = \frac{2}{0.275} = 7.3.$$

where l = length of inner cylinder.

γ = radius of inner surface of outer cylinder = 1.75.
γ' = radius of outer surface of inner cylinder = 0.9.

The thickness of the films of blood on both the inner and outer tube would appreciably diminish the volume of gas and thus increase the relation of area to volume so that the apparatus has probably an actual specific surface of about 10.

Compared with older tonometers on this basis no special advantage has been obtained. The earliest used, Pflüger's, had a specific surface of 3.3 while the latest pattern of Krogh's (leaving aside his micromonometer) has a specific surface of about 30. But it must be here pointed out that the rapidity of diffusion depends not alone on the specific surface, but also on the rate or renewal of that surface.

With each rotation of the tonometer a new surface of blood is presented for diffusion whereby the exchange of gases is greatly facilitated. It would seem desirable then, to employ as a unit in comparing aerotonometers the "specific surface per minute;" that is to say, the surface in square centimeters divided by the volume of gas in cubic centimeters and multiplied by the number of times that surface is renewed per minute.

The tonometer was usually revolving at the rate of 30 revolutions per minute so that the "specific surface per minute" of this apparatus may be put at 300.

It is not easy to calculate the specific surface per minute of Krogh's apparatus but it would probably reach a value of 100, as indicated in the following estimation.

Air space 5–15 cc. say 10.

Rate of flow of fluid 15–44 cc. minutes say 30.

Specific surface 20–33 say 30.

Specific surface per minute—$\frac{30}{10} \times 30\text{-}90$

To ascertain the equilibrium time of the tonometer a series of experiments was undertaken in which a quantity of defibrinated blood was circulated through the apparatus and samples of gas extracted at different times. The perfusion apparatus used was the same as that employed in the experiments on the excised lung (3).

The following experiments will serve to indicate the accuracy of the method and the time taken for equilibrium to be attained.

February 10, 1912. Dog's blood defibrinated. Barometer 755 mm. Hg., Temperature 18°C.

EXPERIMENTAL CONDITIONS AND INTERVALS IN MINUTES	PRESSURE OF GAS IN MM. HG IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm*
Tonometer filled with room-air 0 (circulation established)	−0.0						
10	−0.5	0.19 0.12	0.15	1.1	19.83 19.73	19.78	146.3
15	−1.0	0.20 0.12	0.16	1.2	19.70 19.64	19.67	145.4

February 10, 1912.—Continued.

EXPERIMENTAL CONDITIONS AND INTERVALS IN MINUTES	PRESSURE OF GAS IN MM. HG IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
Tonometer filled with expired air							
0	−0.0						
10	−1.0	0.53 0.44	0.48	3.5	19.44 19.33	19.38	143.2
15	−0 5	0.44 0.40	0.42	3.1	19.20 19.21	19.20	142 2

February 13, 1912. 140 cc. of defibrinated dog's blood. Barometer 765.8 mm. Hg.

EXPERIMENTAL CONDITIONS IN THE CIRCULATION AND INTERVALS IN MINUTES	TEMPERATURE	PRESSURE OF GAS IN MM. HG IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
	deg. C.		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
10	16 8	−2 5	2.85 2.92	2 89	21.7	18.00 17 89	17.94	134 5
15	18 0	−4.0	3.27 3 18	3.22	24 0	17.46 17 54	17.50	130 5
Tonometer filled with expired air								
10	19 7	−4 0	3 65 3 66	3.65	27 2	17.77 17.66	17.70	132.1
15	20 5	−5 5	3 75 3 70	3.72	27 6	17.59 17.64	17.62	130 8
Tonometer filled with expired air								
5	21 8	−1 7	3 90 3 92	3 91	29 1	17 45 17 48	17 47	130 1
10	21 8	−2 5	3 89 3 84	3 87	28 8	16 66 16 64	16 63	123 7

March 20, 1912. 135 cc. of defibrinated dog's blood in the system. Rate of flow 70 cc./minutes. Circulation time once every 2 minutes. Barometer 755 mm. Hg.

EXPERIMENTAL CONDITION AND INTERVAL IN MINUTES	TEMPERATURE	PRESSURE OF GAS IN TONOMETER	SAMPLES OF GAS FROM TONOMETER					
			CO$_2$			O$_2$		
			Analysis	Mean	Tension	Analysis	Mean	Tension
		deg. C.	*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
Expired air into tonometer								
15	22.8	0.5	1.06 1.12	1.09	8.0	17.79 17.83	17.81	130.9
20	22.8	0	1.06 1.01	1.04	7.6	17.78 17.77	17.78	130.7
Expired air into tonometer								
15	21.0	0.5	1.09 1.17	1.13	8.3	16.62 16.78	16.70	123.1
20	20.8		1.34 1.36	1.35	9.9	16.81 16.93	16.87	124.4

April 9, 1912. 225 cc. of defibrinated blood. Rate of flow 100 cc./minutes. Barometer 755 mm.

EXPERIMENTAL CONDITION AND INTERVAL IN MINUTES	PRESSURE OF GAS IN TONOMETER	TEMPERATURE	SAMPLES OF GAS TAKEN FROM TONOMETER					
			CO$_2$			O$_2$		
			Analysis	Mean	Tension	Analysis	Mean	Tension
	deg. C.	*deg. C.*	*per cent*	*per cent*	*mm*	*per cent*	*per cent*	*mm.*
0 (circulation established)								
15	1.0	21.	1.19 1.15	1.17	8.6	16.56 16.41	16.48	121.3
20	1.5	21.	1.12 1.20	1.16	8.5	16.35 16.39	16.37	120.1
Expired air into tonometer								
20	−1.0	21.8	1.02 0.98	1.00	7.3	17.43 17.50	17.46	128.3
25	−1.5	21.2	1.06 0.91	0.99	7.3	17.39 17.36	17.37	127.6
Expired air into tonometer								
15	−1.0	20.6	1.08 1.10	1.09	8.0	15.46 15.53	15.50	114.0
20	−1.5	20.8	1.01 0.98	1.00	7.3	15.57 15.60	15.59	114.6

DISCUSSION

In the experiment of February 10, the sample of gas taken after the blood had been in contact with the gas in the tonometer for 15 minutes showed the same tension as the sample taken after 10 minutes. In the one of February 13, the last set of samples which was taken 5 and 10 minutes after air was expired into the tonometer, differed to an extent greater than could be accounted for by experimental error. The factor of the rapidity with which the blood is circulating would modify the results obtained and in the experiment of April 9, this point was taken into account by measuring the rate of blood flow. The figures obtained in this instance show that when a good rate of flow is obtained, equilibrium is reached within 15 minutes, and probably in 10 minutes as shown in the experiment of February 10.

CONCLUSIONS

A new type of aerotonometer is described in which equilibrium between blood and air was reached certainly in fifteen minutes, and with a good rate of flow in ten minutes.

It possesses the further advantage of providing abundant gas for duplicate analyses.

The credit of designing this apparatus belongs exclusively to the late Prof. T. G. Brodie; my small contribution consisted in carrying out the experiments and the responsibility for the accuracy of the gas analyses rests with me.

In some experiments in which the rate of flow through the system was not observed, equilibrium was not reached in 10 minutes. Several experiments were then undertaken in which the rate of flow was particularly attended to.

The rate of flow was estimated by diverting the flow for a few seconds from the rubber tube where it leaves the tonometer into a graduated cylinder. The time was determined by a stop watch. Several readings were taken, the blood withdrawn being restored to the circulation after each estimation through the funnel. (See description of the artificial circulation in the article "The lung as an aerotonometer.")

BIBLIOGRAPHY

(1) Krogh: Skand. Arch., 1908, xx, 262.
(2) Krogh: Skand. Arch., 1908, xx, 260.
(3) O'Sullivan: This Journal, 1918, xlvii, 143.

THE LUNG AS AN AEROTONOMETER

PAUL M. O'SULLIVAN

From the Department of Physiology, The University of Toronto

Received for publication July 19, 1918

In contemplating some experiments which required that the gaseous tension of the blood in a living animal be quickly and accurately determined, the need of an efficient aerotonometer was encountered.

From a priori considerations an excised lung would seem to afford an ideal aerotonometer on the supposition that the alveolar surface of an excised lung, when perfused, acted solely as a physical structure. Here the blood is spread out in an exceedingly fine layer, approximating to the thickness of one blood corpuscle and exposed to the process of diffusion with the air in the alveoli. The area of surface of exposure of the blood, measured in square centimeters is more than two hundred times the volume of air enclosed in the alveoli, measured in cubic centimeters. Moreover in a perfused lung this surface of blood is rapidly renewed.

To test this hypothesis the following experiments were performed. *In the first series of experiments* the inflated excised lung was perfused through its vessels with defibrinated blood and at various intervals a sample of gas was withdrawn from the trachea for analysis. If successive samples of gas showed the same composition, the gaseous pressure remaining constant, it would indicate that equilibrium between the gases in the blood and the gases in the alveoli had been attained.

An inflated human lung has a capacity of about 3700 cc. and a surface available for exchange of gases of about 90 sq. m. This would mean a specific surface (area of exchange surface in square centimeters divided by volume of gas enclosed in cubic centimeters) of about 240.

METHOD

In these experiments a dog was bled to death; the blood was whipped and filtered through glass wool. The thorax was opened and cannulae inserted into the pulmonary artery, before its bifurcation and into the left auricular appendix. A strong ligature was tied around the heart so as to close the auricular-ventricular openings and thus the systemic circulation was excluded. A glass tube was inserted into the trachea and was connected by rubber tubing with a mercury receiver. By raising and lowering this receiver the air in the lung alveoli was mixed with the dead space air. Gas samples could be extracted from a side tube.

Figure 1 is a diagram of the artificial circulation through which the defibrinated blood was perfused, the blood being introduced through the funnel *X*, which is closed by a weighted mercury stopper. About 250 cc. of blood are required to fill the system of tubes and the blood vessels of the lungs. A mean air pressure is transmitted to a rubber bag, *B*, which acts upon the blood contained in the arterial glass reservoir, *R1*. This air pressure is obtained by attaching a tube from the water tap and directing it into a large 10 liter jar, *F*, tightly corked. The water runs away from the large orifice at the bottom of the jar, and this exit is provided with rubber tubing and a screw clamp. By tightening this screw the rate of outflow may be lessened and thus the pressure of air in the jar above the water is increased. On the path of the tubing between the jar and the arterial reservoir are two side tubes. One, *D*, serves as a manometer; the other side tube, *E*, dips into the long cylinder containing mercury and by altering its depth in the mercury the pressure in the system is regulated. The blood is driven into the arterial reservoir, *R1*, by the pump, *C*, a rubber bag alternately compressed and relaxed by a crank movement, *K*, operated by a motor. The pump is provided with a ground glass valve, *V*, of well-known de-

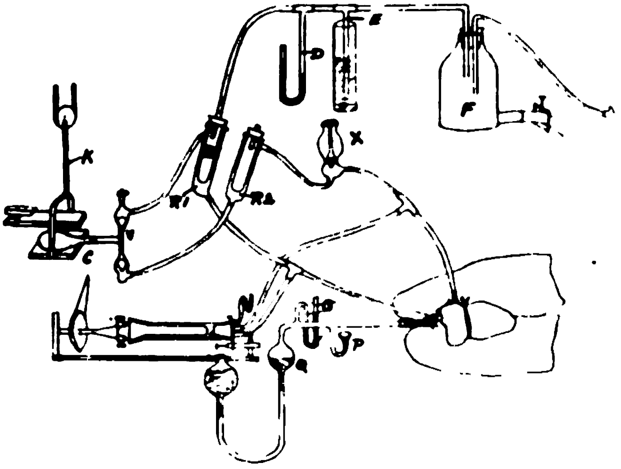


Fig. 1.

sign, which insures that the direction of flow shall be toward the arterial reservoir. From the arterial reservoir the blood is driven through rubber tubing into the pulmonary artery and collected again by the cannula in the left auricular appendix and sent into another, the venous reservoir, *R2*. The venous reservoir is a thick glass tube exactly similar to the arterial one, provided with an elastic bag by means of which the venous pressure may be altered by blowing into the tube inserted into the cork which closes the open end. The blood from the lungs enters the venous reservoir at the corked end of the tube and leaves the reservoir from the opening at the opposite end. From this point the blood is returned to the valve by tubing and the circulation is completed. The return flow is assisted by gravity, the valve being placed lower than the venous reservoir. In the first series of experiments the lung was perfused with defibrinated dog's blood.

The lungs are inflated and the trachea is attached by rubber tubing to the mercury receiver, *Q*. Before taking the sample, the air in the trachea, rubber tubing and mercury bulb (dead space air) is thoroughly mixed by raising and lowering the mercury receiver. A manometer, *G*, is also attached to this tube which gives the pressure of air in the lungs at the time sample is taken. In some cases the entire mechanism was enclosed in a thermostat and the experiment conducted at about body temperature. Air samples are withdrawn through the mercury seal, *P*, the tip of the gas-sample pipette fitting into a small piece of rubber tubing at the bottom of the little cup attached to the T-piece, which is filled with mercury. When a sample is taken it is sealed off with mercury and may be analyzed at leisure. Analyses in duplicate are made and from them the gaseous tensions of the gases in the lung are estimated. For example, supposing the barometer stands at 757 mm., the temperature of the blood in the system is 36°C., the manometer attached to the tube to the trachea shows a pressure of 2 mm. An analysis of a sample taken shows:

$$CO_2 = 3.24 \text{ per cent}$$
$$O_2 = 15.75 \text{ per cent}$$

then the total pressure of the gases in the lung = 757.0 − 2 = 755.0 mm.
deducting pressure of aqueous vapour at 36°C. = 44.2 mm.
then the pressure due to CO_2 plus O_2 plus N_2 = 710.8 mm.

$$\text{therefore tension of } CO_2 = \frac{3.24}{100} \times 710.8 = 23 \text{ mm. Hg.}$$

$$\text{tension of } O_2 = \frac{15.75}{100} \times 710.8 = 112 \text{ mm. Hg.}$$

and the nitrogen is obtained by deducting the sum of these two from the total pressure less aqueous tension. Samples are taken at 1, 2, 3, 4 and 5 minutes. When successive samples show the same tension, then it is assumed that equilibrium has been reached.

RESULTS

The following figures will show some of the results obtained.

March, 1911. Blood obtained from dog. Thorax in thermostat at 36°C. Barometer 755 mm. Hg.

TIME	OBSERVATIONS	INTERVAL IN MINUTES	GAS—SAMPLES FROM LUNGS					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	mm. Hg.	*per cent*	*per cent*	mm. Hg.
10.56	Commenced mixing alveolar air with air in dead space							
10.59	Sample 1 withdrawn......	3	3.17 3.31	3.24	23	15.88 15.63	15.75	112
11.05	Sample 2................	9	3.19 3.11	3.15	22	15.32 15.57	15.45	110
11.10	Sample 3.	14	2.89 2.88	2.88	20	15.23 14.94	15.08	107

March 7, 1911. Blood obtained from dog ♀ 15 kgm. Thermostat 36°C. Barometer 755 mm.

TIME	OBSERVATIONS	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	mm. Hg	*per cent*	*per cent*	mm. Hg.
12.29	Circulation established							
12.40	Commenced mixing gases							
12.41½	Sample 1	1½	4.53 4.32	4.42	31	13.89 14.09	13.99	99
12.46	Sample 2 ...	6	4.56 4.64	4.60	33	13.63 13.68	13.65	97
12.52	Sample 3	12	4.99 4.90	4.95	35	13.31 13.25	13.28	94
1.08	Sample 4	38	5.42 5.57	5.48	39	12.58 12.38	12.48	91

March 10, 1911. Blood from dog ♂ temperature 30°C. Barometer 755 mm. Hg.

DATE	OBSERVATIONS	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS					
			CO₂			O₂		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	*mm.* Hg.	*per cent*	*per cent*	*mm.* Hg.
2.56	Circulation established							
3.00	Sample 1	4	1.83 1.89	1.85	13	16.25 15.92	16.08	120
3.02½	Sample 2	6½	1.47 2.10	1.78	13	16.54 16.22	16.38	120
3.32	Sample 3	36	1.85 1.83	1.84	13	14.89 14.77	14.83	108

June 13, 1911. 225 cc. of blood from dog 14 kgm. Barometer 755 mm. Hg.

TIME	OBSERVATIONS	LUNG PRESSURE	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG					
					CO₂			O₂		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm. Hg.	*deg. C.*		*per cent*	*per cent*	*mm.* Hg.	*per cent*	*per cent*	*mm.* Hg.
12.00	Circulation established									
12.10	Sample 1	9	28.5	10	3.87 3.85	3.86	28.4	16.00 15.87	15.94	117
12.30	Sample 2	8	30.0	30	4.02 3.98	4.00	29.3	14.99 14.86	14.93	109
1.04	Sample 3	6	32.5	64	4.07 4.12	4.09	29.6	13.77 13.65	13.71	99
1.35	Sample 4	5	34.5	95	4.18 4.25	4.21	30.3	12.27 12.31	12.29	88
2.17	Sample 5	5	34.0	137	4.16 4.05	4.10	29.5	9.92 10.04	9.98	72

From these experiments it will be seen that samples taken within ten minutes of each other agree fairly well whereas a sample taken after an interval of twenty minutes or thirty minutes after a given sample shows an obvious decrease in oxygen tension. This may be accounted for by the oxygen metabolism of the living elements of the blood. For example in the experiment of June 13, the oxygen tension fell from 117 mm. to 72 mm. in 127 minutes. At the CO_2 tension of 30 mm. this corresponds to a fall in oxygen content from 98 per cent saturation to 94 per cent saturation (1). There were 225 cc. of blood in the apparatus. Assuming that 100 cc. of dog's blood when saturated contain 18 cc. of oxygen, and neglecting the amount physically dissolved in the plasma, this means an oxygen consumption of 0.000026 cc./gm./min. Krogh's figures (2) for the oxygen-metabolism of rabbit's blood (hirudinized) vary from 0.00046–0.00075 cc./gm./min. at body temperature.

Another experiment in which this feature was especially noted is here given.

April 10, 1911. 225 cc. of blood (dog). Temperature 31°C. Barometer 755 mm. Hg.

	TIME IN MINUTES	AIR SAMPLES FROM LUNG					
		CO_2			O_2		
		Analysis	Mean	Tension	Analysis	Mean	Tension
		per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
Sample 1, 7 mm.	0	3.36	3.37	24.6	14.36	14.30	104.5
		3.39			14.24		
Sample 4, 7 mm.	113	3.54	3.55	25.9	7.84	7.50	54.8
		3.55			7.16		

At a temperature 31°C. $\alpha = 0.0258$ (α = coefficient of absorption of oxygen dissolved in water).

At 104 mm. of O_2 (CO_2 tension 25 mm.) 225 cc. of blood contain

$$\frac{25.8 \times \frac{14.30}{100} + 180 \times \frac{98}{100} \times 225}{1000} = 40.52 \text{ cc. of } O_2$$

At 55 mm. of O_2 (CO_2 tension 26 mm.) 225 cc. of blood contain

$$\frac{25.8 \times \frac{7.50}{100} + 180 \times \frac{85}{100} \times 225}{1000} = 34.85 \text{ cc.}$$

225 cc. of blood use up 5.67 cc. of O_2 in 113 minutes -about 0.00022 cc./gm./min.

With increase in temperature of about 10°C. the oxygen consumption increased about tenfold. In all cases the fall in oxygen tension can be accounted for by the oxygen metabolism of the living elements of the blood.

In the second set of experiments one of Krogh's aerotonometers was inserted into the circulation in parallel with the lung. This apparatus takes from twenty to thirty minutes to attain equilibrium (3). Samples were taken from the lung as nearly as possible synchronously with the samples taken from the aerotonometer.

The following results were obtained.

March 24, 1911. Blood from Dog ♂ 21 kgm. Barometer 755 mm. Hg. Temperature 30°C.

TIME	OBSERVATIONS AND LUNG PRESSURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
1.00	Circulation established							
1.04	Sample 1, 6 mm..........	4	2.23	2.19	16.0	18.14	18.23	133
			2.15			18.32		
1.57	Sample 2, 6 mm..........	57	3.50	3.47	25.4	15.61	15.65	114
			3.34			15.68		
2.10	Expired air into tonometer							
2.18	Added 1 cc. of 10 per cent NaF							
2.54	Sample 3, 6 mm..........	114	4.08	4.06	29.7	12.98	13.09	96
			4.04			13.16		
3.00	Aerotonometer sample, 5 mm..................	120		4.09	29.4		12.88	93

April 10, 1911. Dog ♂ 15 kgm. 225 cc. blood. Temperature 30°C. Barometer 755 mm. Air samples from lung and tonometer.

12.05	Circulation established. Expired air into tonometer							
12.30	Lung sample, 7 mm.......	25	3.21	3.46	25	10.81	11.09	82
			3.71			11.37		
12.48	Tonometer sample, 0 mm.	43		4.28	31		12.33	89
1.20	Expired air into tonometer							
1.35	Lung sample, 7 mm.......	90	3.48	3.57	26	8.55	8.58	63
			3.66			8.61		
1.45	Lung sample, 7 mm.......	100	3.54	3.55	26	7.84	7.50	55
			3.55			7.16		
1.50	Tonometer sample, 0 mm.	105		4.82	35		8.10	59

May 12, 1911. Dog ♂ 25 kgm. 225 cc. blood. Temperature, 33°C. Barometer 747 mm.

1.09	Circulation established. Expired air into tonometer							
1.35	Lung sample, 15 mm......	26	2.98	2.81	20	13.52	13.61	99
			2.65			13.71		
1.40	Tonometer sample, 0 mm.	31	3.54	3.48	25	13.80	14.15	100
			3.43			14.51		
1.50	Expired air into tonometer							
2.23	Lung sample, 13 mm......	33	3.94	4.03	28	11.16	11.12	80
			4.12			11.09		
2.28	Tonometer sample, 5 mm....................	38	3.67	3.67	26	12.83	12.78	98
			3.68			12.74		

March 17, 1911. Dog ♂ 20 kgm. Thermostat 27°C. Barometer 756.8 mm.

TIME	OBSERVATIONS AND LUNG PRESSURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
			CO_2			O_2		
			Analysis	Mean	Tension	Analysis	Mean	Tension
			per cent	*per cent*	mm.	*per cent*	*per cent*	mm.
12.01	Circulation established.							
12.07	Expired air into tonometer							
12.11½	Lung sample 1, 7 mm.....	10½	3.24 3.52	3.38	25	13.29 13.15	13.22	97
12.15	Added 2 cc. of 10 per cent NaF							
12.19	Lung sample 2, 7 mm....	18	3.45 3.41	3.43	25	13.00 12.97	12.99	96
12.40	Tonometer sample 0 (33′)	39	3.27 3.90	3.58	26	15.03 14.11	14.57	
12.42	Expired air into tonometer							
12.43½	Lung sample 3, 7 mm.....	42½	3.46 3.30	3.38	25	12.30 12.12	12.21	90
1.16	Aeronometer sample, 0 mm (36)..	70	3.94 3.28	3.61	26	10.64 10.87	10.75	78

These experiments show a general correspondence between the tension of the gases in the lung and in Krogh's tonometer. For example:

April 10, 1911. After 25 minutes interchange of gases in the blood and in the lung and tonometer, the lung showed a CO_2 tension of 25 mm. Krogh's apparatus taken 18 minutes later 31 mm., while the oxygen values were 82 mm. and 89 mm. respectively. In the same experiment an hour later the results were as follows:

	CO_2	O_2
Krogh's......	35	59
Lung..........	26	55

In an experiment of *March 24, 1911*, a sample of gas from the lungs was taken 34 minutes after introducing air into the tonometer, and 6 minutes later a sample was taken from the tonometer. The analysis shows that the lung and tonometer give the same result within the limits of experimental error. In this experiment the lung had been reduced to a physical structure by killing the cells by the addition of sodium fluoride to the blood. Evidently the lung acts as a tonometer independently of any vital activity of the alveolar cells.

Many difficulties were encountered in using Krogh's aerotonometer. Especially was it found difficult to obtain a good film of blood since the blood ran down the tube in streaks. Another difficulty was that only a small quantity of gas was available for analysis.

In the final series of experiments a new type of aerotonometer was employed, designed by Doctor Brodie, which affords plenty of gas for duplicate analyses and in which the blood is spread out in an even film, which is constantly renewed by the rotation of the tonometer (4). This apparatus is shown connected in parallel with the lung in the figure.

The equilibrium time of this tonometer has been ascertained to be ten minutes with a circulation time of once every two minutes, which is comparable to the rate of flow through the lungs in living animals to fifteen minutes in a circulation time of once every three or four minutes. The following results were obtained:

December 29, 1911

TIME	OBSERVATIONS	LUNG PRESSURE	TEMPERATURE	INTERVAL IN MINUTES	AIR SAMPLES FROM LUNG AND TONOMETER					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm.	*deg. C.*		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
10.40½	Circulation established									
10.42	Lung sample.........	0	17	1½	0.82 0.62	0.72	5	17.00 17.28	17.14	127
10.49	Tonometer sample...	0	17	8½	1.04 0.76	0.90	7	17.63 17.53	17.58	130
10.51	New air introduced into tonometer									
11.06	Lung sample.........	0	17	15	1.12 1.00	1.06	8	16.28 16.36	16.32	121
11.13	Tonometer sample...	0	17	22	1.18 1.10	1.14	8	17.88 17.76	17.82	132

The blood in this experiment was obtained from a dog used in the morning. The blood and thorax had been kept in a refrigerator.

April 12, 1912. Lung taken from a dog on April 9. Saline was run in as the animal was bled. Subjected to artificial ventilation with formalin vapor for 5 hours. Lungs kept on ice. Blood taken from dog about 200 cc.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS AND TONOMETER						RATE OF FLOW
					CO_2			O_2			
					Analysis	Mean	Tension	Analysis	Mean	Tension	
		mm.	deg. C.		per cent	per cent	mm.	per cent	per cent	mm.	cc./min.
3 51	Circulation established										
4 01	Lung sample	9	21.5	10	0.93 1.07	1.00	8	18.50 18.38	18.44	137	
4.06	Tonometer sample	−5	21.5	15	1.55 1.47	1.51	11	17.19 17.09	17.14	125	10
4 08	Expired air into tonometer										
4.19	Lung sample	9	20.2	11	1.32 1.16	1.24	9	18.41 18.37	18.39	137	
4 23	Tonometer sample	5	20.2	15	0.77 0.55	0.66	5	18.69 18.63	18.66	137	60

April 20, 1912. Fresh lung of cat; about 100 cc. of cat's blood made up to 200 cc. with 0.85 per cent saline. Rate of flow in lung 100 cc./min. Circulation time 1 every 2 minutes. Rate of flow in tonometer 60. cc./min. Circulation time 1 every 3.3 minutes. Barometer 755.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNGS AND TONOMETER					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm.	deg. C.		per cent	per cent	mm.	per cent	per cent	mm.
5.45	Circulation established									
5 55	Lung 1	2	16.5	10	1.56 1.48	1.52	11	18.18 18.22	18.20	135
6 05	Tonometer 1	−5	15.8	20	1.25 1.31	1.28	10	18.98 18.91	18.95	140
6.06	Air into lungs and tonometer									
6 21	Lung 2	15	15.5	15	0.80 0.69	0.75	6	18.88 18.82	18.85	143
6 23	Tonometer 2	+5	15.3	16	1.05 0.92	0.99	7	19.24 19.16	19.20	142
6 28	Lung 3	7	15.3	22	0.55 0.54	0.55	4	18.97 18.92	18.94	142
6 29	Tonometer 3	+8	15.2	22	0.60 0.53	0.56	4	18.74 18.69	18.72	137

In these later experiments the carbon dioxide tension was very low. In the following experiments the blood was saturated with CO_2 before it was introduced into the circulation.

January 7, 1913. 190 cc. of blood from a dog 7 kgm. Barometer 757. Aerotonometer revolving 36/min. Arterial pressure 60/mm.

TIME	OBSERVATIONS	PRESSURE OF GAS IN LUNG	TEMPERATURE	INTERVAL IN MINUTES	GAS SAMPLES FROM LUNG AND TONOMETER					
					CO_2			O_2		
					Analysis	Mean	Tension	Analysis	Mean	Tension
		mm.	*deg. C.*		*per cent*	*per cent*	*mm.*	*per cent*	*per cent*	*mm.*
1.32	Circulation established									
	Rate of flow: Tonometer 48 cc./min.									
	Rate of flow: Lungs 34 cc./min.									
1.47	Lung sample 1........	10.5	18.3	15	5.93	5.96	44.8	15.74	15.60	117.0
					6.00			15.49		
1.51	Tonometer sample 1...	1.0	18.3	19	6.20	6.33	46.8	16.04	16.00	118.4
					6.46			15.96		
1.53	Expired air into tonometer									
1.54	Expired air into lungs									
2.08	Lung sample 2........	10.5	18.2	14	3.69	3.78	28.4	17.30	17.32	130.2
					3.87			17.34		
2.09	Tonometer sample 2..	1.0	18.2	16	3.75	3.78	28.0	16.99	16.92	125.3
					3.81			16.84		
2.25	Expired air into lungs and tonometer									
2.40	Lung sample 3........	10.5	18.6	15	2.83	2.91	21.9	18.12	18.09	135.9
					2.98			18.05		
2.44	Tonometer sample 3..	1.0	18.6	19	3.12	3.11	23.0	17.57	17.64	130.5
					3.11			17.71		
	Rate of flow: Tonometer 50 cc./min.									
	Rate of flow: Lungs 20 cc./min.									
2.50	Expired air into tonometer									
2.51	Expired air into lungs									
3.06	Lung sample 4........	+28.0	17.8	15	2.00	1.98	15.2	18.42	18.35	141.4
					1.96			18.29		
3.09	Tonometer sample 4...	− 1.0	17.8	19	1.92	1.97	14.6	18.10	18.23	135.1
					2.02			18.36		

DISCUSSION

In the experiment of December 29, after the gases in the blood had been in contact with the gases in the tonometer and with the gases contained in the freshly excised lung for a period of from ten to twenty minutes, a close correspondence in the gaseous tension was obtained. In the experiment of April 12, a similar agreement was obtained after allowing an interchange of gases for fifteen minutes, but in this instance the alveolar cells of the lung had been killed by ventilating the lung previously with formalin vapor for several hours. The experiment of April 20 shows a close correspondence after fifteen minutes exposure.

Four sets of satisfactorily corresponding tensions were obtained in the experiment of January 7. The gases in the lung undoubtedly reach equilibrium in about one minute but as ten to fifteen minutes must be allowed to be confident that equilibrium has been attained in the Brodie tonometer, the samples were taken at longer intervals.

These experiments show that the lung acts as a tonometer either freshly excised or after having been killed by chemicals.

The accuracy of the determination of gaseous tension as shown by the duplicate analyses does not permit a comparison beyond a decimal point. In the earlier experiments an error of 5 per cent must be allowed but after long practice duplicate analyses were obtained which showed a variation of not more than ± 0.05.

In making use of an excised lung to determine the gaseous tension of the blood of a second animal, it was found that the blood clotted much more readily in lungs which had been fixed by formalin than it did in fresh lungs. Moreover in using a cat's lung to determine the tension of dog's blood, clotting was encountered. The best results were obtained when using a puppy's lung to determine the tension of dog's blood.

CONCLUSIONS

The excised lung, either freshly prepared or after treatment with sodium fluoride or formalin, when perfused with defibrinated blood, acts solely as a physical surface and may be used as an aerotonometer. It affords sufficient surface so that equilibrium between the gases in the blood and the gases in the alveoli is attained in at most one and one-half minutes, which is the time required to take a sample.

It was the late Prof. T. G. Brodie who originated the conception that the lung might be used as an aerotonometer and his kindly counsel in

directing my work has been the source of any value that the research may contain.

BIBLIOGRAPHY

(1) Bohr, Hasselbalch and Krogh: Skand. Arch., xvi, 409.
(2) Krogh: Skand. Arch., 1910, xxiii, 193.
(3) Krogh: Skand. Arch., 1908, xx, 262.
(4) O'Sullivan: This Journal, 1918, xlvii, 137.

ON THE HEAT LIBERATED BY THE BEATING HEART

SECOND COMMUNICATION

CHARLES D. SNYDER

From the Department of Physiology, The Johns Hopkins University

Received for publication July 19, 1918

Having shown in a former communication (1) that the chelonian heart during its beat undergoes a measurable change of temperature, it seemed desirable to obtain more objective records of this change, for it will be remembered that the method of registration of the galvanometer response to the rise and fall of temperature consisted of a mechanical lever operated by the observer. If photographic registration could be substituted for the hand-operated recorder this subjective element could be removed.

The use of the Einthoven instrument on account of its fitness for photographic registration had already been considered, but that instrument also has its disadvantages as will appear in the following.

The fiber I happen to have is a gilded quartz fiber of 5400 ohms resistance. The galvanometer is the small Edelmann model. To give an idea of its availability for recording thermo-electric effects from a turtle's heart beating say, at a rate of five per minute, the following observations will serve.

With the tension of the thread set at 15, the calibrating current produced a deflection of 5 mm. of scale, with the tension set at 16, the deflection to the same current was increased to 22.5 mm. of scale. The periods of the instrument at the two tensions of the thread, however, were 0.7 and 2.7 seconds respectively. The calibrating current was of constant strength, permitting 4.6×10^{-9} ampere to pass through the galvanometer. With the clasp-thermopile in series with the galvanometer the difference of temperature between the warm and cold junctions necessary to create an amperage equal to the calibrating current would have to amount to nearly 0.0156°C. The difference of temperature that could be registered by 1 mm. deflection of the galvanometer thread set at the two tensions named above would then be nearly 0.003° and 0.0007° respectively.[1]

In my former study it was shown that a rise of about 0.0013° may be expected per beat of the turtle's ventricle. With the tension of the thread in the galvanometer set at the greater of the two sensibilities one would expect a deflection of 2 mm. per beat, provided further that the duration of the beats is at least twice the period of the galvanometer. The period being 2.7 seconds the duration of beat should be reduced to 5.4 seconds. If the duration of the beat should happen to be 5 instead of 5.4 seconds it is clear that a slight increase in tension on the thread will bring its period within the limits prescribed without reducing the sensibility greatly.

---

[1] For this calculation the reader is referred to the formula given on page 424 of my first communication (1). The derivation of that formula was not given there. For purposes of checking up results it is here added:

Let I be the strength of thermo-electric current produced by the thermopile in use when a difference of 1°C. obtains between the two sets of junctions and when the current passes through the thermopile (of r_1 resistance) and the galvanometer (of r_2 resistance) in series. Then when the calibrating current (in the present case 4.6×10^{-9} amp.) produces a deflection of m divisions of scale the rise of temperature necessary to give an equal deflection will be i/I, where i represents the amperage of the calibrating current. This value then divided by the number of scale divisions deflection to the calibrating current, m, will give the difference of temperature represented by 1 scale division deflection, Δt.

In the present experiments I has the value, $\frac{30 \times 53 \times 10^{-6}}{5400 + 11.32}$, and i has the value, 4.6×10^{-9}. Where i yields 22.5 as a value for m, Δt equals 0.00069; with 5 as a value for m, Δt equals 0.003°C.

Combining the operations into one generalized equation we have the formula,

$$\Delta t = \frac{r_1 + r_2}{n \cdot p \cdot m} \cdot i$$

as given in my former paper, in which n represents the number of elements and p the thermo-electric power of one element, in the thermopile used.

To complete the account it should be added that the calibrating current was derived from a normal cell of 1.0187 volts with a constant resistance of 38,220 ohms connected in series to a shunt of 1 ohm and the galvanometer of 5400 ohms arranged in parallel. The total resistance of the circuit thus remains practically at 38,220 ohms and the total amperage is 2.5×10^{-5}. The strength of current passing through the galvanometer thus is tot. amp. times the product of the reciprocals of the parallel resistances, or $i = 4.6 \times 10^{-9}$ ampere.

Further it should be stated that the deflections of the galvanometer are taken to be a linear function of the strength of current passing which for the ranges of current employed is practically the case.

With the foregoing discussion as to what may be expected of the Einthoven instrument in mind, I will now proceed to a brief description of the experiments and their results. For details of method and apparatus not here given the reader is referred to the former study.

Experiment of March 15, 1918. A turtle's heart was suspended in the moist chamber with the clasp-thermopile in place. The action of the heart on the thermopile junctions was observed for some minutes. No change in position between heart wall and junctions took place and it was assumed that this condition continued during the experiment. Indeed one can be certain, after some experience, from the character of the galvanometer deflections and especially from their photographs just when the contacts remain in ideal relation. The moist chamber having been supplied with ice the temperature soon fell to about 12.5°C. and remained there during the taking of records. The rate of the beating ventricle was thereby slowed down to about 12 beats per minute. The mechanical movements of the ventricle walls were recorded by an isometric lever that had been carefully calibrated. A time trace was provided for by a Jaquet marking fifths of a second. The sensibility of the galvanometer was noted just before and after each photograph taken. The rate of the moving film and its length permitted a record of two or three complete heart beats to the photograph. Memoranda and analysis of two of the records so taken may be here reproduced.

Photo-record 1. Calibrating current just before gives 15 mm., just after gives 12.5 mm. deflection of thread. Distance of optical projection, 119 cm. Distance of recording tip of mechanical lever from camera shutter, 13 cm. Measurement of the curves in the photograph shows a maximum excursion of the mechanical lever of 13.0 mm.; a maximum deflection of the galvanometer thread of 1.9 mm., which is monophasic and single for the whole of a cardiac cycle and begins nearly a second after the beginning of the rise of the myogram curve The duration of a single heart beat is 5 seconds, of the systole (ascending limb of myogram) 2.2 seconds, diastole and pause 2.8 seconds. The measurements are the same for all the heart beats in the record.

Photo-record 2. Deflection of galvanometer to calibrating current remains constant, 15 mm. of scale. Other conditions the same as for the preceding record. Measurements from the photograph: maximum excursion of mechanical lever, 13.5 mm.; maximum deflection of thread, 2.1 mm.; initial rise of galvanometer deflection 0.5 second after initial rise of mechanical lever; duration of each beat, 5.4 seconds; of systole, 2.0 seconds; of diastole and pause, 3.4 seconds.

At the end of the experiment the ventricle free from large vessels, auricles and blood was found to weigh 3.6 grams

Calculations From the calibration data of the mechanical lever (not here reproduced) and from the data of the observed optical projection one can determine the actual shortening of the ventricle wall and the maximum tension developed during each heart beat. From the data on the electrical and thermo-electrical quantities involved, as given above, one can calculate the changes of temperature represented by the galvanometer deflections.

The results of the corrections for the mechanical curve show an actual maximum shortening of the muscle of 1.18 mm. and a maximum tension developed during each systole of 13.7 grams for each beat in the first record and 14.3 grams for each beat in the second record. The sensitivity of the galvanometer was not the same for both records,—not more than 13.75 mm. deflection can be allowed for the first record, as against 15 in the second. So while there appears to be considerable difference in the rise of temperature in the two records actual calculation, according to the method given above, shows a rise of temperature during each heart beat in the first record to be about 0.00215°C. and in the second record to be about 0.00218°C. This represents a heat production in each case, taking the specific heat of muscle to be 0.825, of about 0.0007 calorie per gram of ventricle per beat.

Experiment of May 3, 1918. In this experiment the method and instrumentarium are all the same except that a Rohde (2) heart-bag was used instead of the isometric muscle lever to record the mechanical events. The bag fixed at the end of a cannula was inserted into the ventricle chamber through the aortic arches, the intervascular septum and the ventricular septum having been previously slit down sufficiently to make a single passage way and chamber. The bag was made to communicate with a Hürthle membrane manometer. This manometer was calibrated just before the experiment so that the excursions of the recording tip could be converted into terms of a column of water pressure. In this experiment 0.5 mm. excursion of the lever tip was equal to a difference of 10 mm. change in the height of a column of water. The recording tip of the manometer lever was so adjusted as to have its shadow fall across the aperture in the camera in a position appropriate to the shadows of the other recording levers.

The first photo-record in this experiment was not brought to a successful finish. The second was more successful and the data for this are as follows:

Photo-record 2. Initial tension in heart-bag, that is, diastolic pressure, 25 cm. water. Temperature of moist chamber, 16.5°C. Deflection of galvanometer thread to calibrating current, 20 mm. of scale.

In the photograph the duration of the heart beat is about 9.6 seconds (partial block due to slitting ventricular septum?). The duration of the systole, that is of the ascending limb of the manometer lever, is about 2 seconds. The manometer lever shows in each case an excursion of 3 mm. or a rise of tension during systole of 6 cm. water-column.

The galvanometer thread shows a deflection of 2 mm. for each heart beat which I calculate to represent a rise of temperature of 0.00156°C. The heart was not weighed in this experiment but since the turtles used were nearly the same size one can make an estimate of the ventricle's weight. If that is taken as 3.6 grams then the heat production was 0.000526 calorie; if the weight is taken as 3.0 grams then the heat production was 0.000531 calorie per gram ventricle per beat.

Explanatory note to the figures in the folder. Before passing on to any general discussion a word explanatory to the figures as they appear in the attached folder should be added. The figures in each case should be read from left to right. The nethermost tracing in each figure is that of the time marking fifths of seconds. The heavier of the two tracings in the body of each figure is that of the mechanical lever (whose ascending limb is caused by the systole of the beat), the lighter one will be recognised as that of the galvanometer thread.

Figure 1 is photo-record 1 of the experiment of March 15. Here the mechanical curve represents an isometric muscle lever attached to the frenum of the heart.

Figure 2 is photo-record 2 of the experiment of May 3. Here the mechanical curve represents the excursions of a Hürthle manometer lever, hence the intraventricular pressure changes or the "pulse pressure." The ascending limb again represents the systole of the beat. In this experiment while the ascending limb of the manometer lever occurs during the heart's systole, the initial rise of the curve of this lever in the photo-records cannot be taken as the point of beginning rise of tension. The initial tension was fixed at 25 cm. The early stages of systole during which this amount of tension is developed receive no indication in the record. No definite statement therefore can be made about the duration of the systole.

The fine horizontal and vertical straight lines found in the photo-records will aid the eye in comparing synchronous events at any point desired.

DISCUSSION

1. The initial heat production in the cardiac cycle. The temporal relations between the initial rise of temperature and the initial shortening of the heart musculature involves one of the most important theoretical points and may be considered first.

The time required for the conduction of heat from the tissue to the thermal junctions of the recording apparatus has already been discussed in my former paper. It was there pointed out that the cooled and blocked turtle's ventricle was a specially suitable preparation for the study of heat production in muscle. It was pointed out that the probably long latent period to the inner stimulus (estimated to be from 0.3 to 0.5 of a second) gave an abundance of time for the conduction of heat if any measureable amount were formed during this period. In that study no evidence appeared of heat production during the

latent period of the muscle contraction or before actual rise of tension occurred in the muscle. The records obtained in the present study, where an isometric lever of small initial tension was used, are similar in this respect to those of the former study. No evidence of pre-systolic heat production appears. In the experiment of May 3, however, where a high initial tension was placed upon the heart-walls it would appear at first sight that heat is liberated during the pre-systolic period (fig. 2 of the folder). Here seems to be a contradiction. But when one reflects upon the conditions of the two experiments this contradiction disappears.

In the first place one must consider the difference in the initial tensions placed upon the muscle in the two experiments. In the first case the initial tension was just enough to balance the weight of the heart, in the second case no weight was needed to balance the weight of the heart but nevertheless its walls were kept distended by the pressure of a column of water 25 cm. in height during diastole. This initial tension was selected by a preliminary trial which showed that no greater tension could be placed on the relaxed muscle and still allow one to get a recordable response of the manometer lever during systole. The tension allowed an increase during systole of 6 cm. in the water column. But before the height of the column could be increased the heart wall had to develop an internal tension from 0 to 25 cm. of water. It is during this period that the record shows no mechanical response to the rising tension. It thus fails to record the early stages of systole. The ascending limb of the galvanometer response in the photograph therefore is initiated before that of the mechanical lever, not because heat production begins before rise of muscular tension but because the mechanical lever fails to record the initial rise of tension.

It may be stated that the experiment of May 3 was only the beginning of a series in a study on the relation of heat quantities and tensions in muscle contractions.

One other important correction should be kept in mind in comparing the mechanical and thermal curves in the photographs. In both experiments the latency of the former is doubtless much greater than the latency of the latter. The latencies have not been actually measured but if their ratios are as we may expect, then supposing the two events to arise within the tissue simultaneously, the mechanical curve would have its beginning in the photograph at a point subsequent to that of the thermal curve by difference of time equal to the difference of their latencies. Now the reverse order of events appears in the records unless one increases the latency of the mechanical event artificially, that is, by raising the initial tension to a point approximating the maximum tension developed.

Summing up, I conclude that the thread galvanometer gives no evidence that heat is produced in the turtle's heart muscle during the pre-systolic period, or during the period that may be called the latent period of contraction or before there occurs within the tissue a rise of tension. This agrees with the conclusion arrived at, using the Paschen galvanometer.

2. Velocity of heat production. As to rate at which heat is liberated at successive intervals of time the records from the Einthoven galvanometer are unsatisfactory. This is due to the small total excursion of the thread which, while great enough to admit of accurate measurement of total rise of temperature over a period of two seconds, does not admit of a measurement of partial rises over periods say, of tenth of seconds. As far as one can judge by mere inspection however the character of the curves in this study is quite similar to that of the curve obtained from the Paschen galvanometer. The rise of temperature proceeds at first slowly then with increasing velocity and finally changes again to a slower rate just before reaching the maximum rise. Indeed the turning point in the curve may be looked upon as being due not to an abrupt cessation of heat liberation but rather to a reduction in the velocity of its liberation to a point where it no longer balances the loss of heat by conduction, etc. For the time of return to null point, that is, of cooling exceeds the period of the galvanometer.

3. Maximal, mechanical and heat effects. It will be noted that the maximum point in the galvanometer curve in the photographs occurs at a point subsequent to the maximum point of the mechanical curve. This delay in the galvanometer maximum can only be explained if we take into consideration the period of the galvanometer. This point was pretty fully discussed in my former paper and need not be gone into again. It is a noteworthy fact however that the delay in the two maxima is in the present study, as in the former, equal nearly to the period of the galvanometer. Parenthetically it may be well to point out again the fact that one has not gained anything in the way of period, the Einthoven being as slow almost in its period as the Paschen. In the curves of figure 1, counting between mid-points of the crests, the delay is a little more than one second; in those of figure 2, a little less than two seconds. Turning to the protocols one notes a sensitivity of 13.7 mm. for the deflection in the curves of figure 1 as against 20 mm.

for the deflections in figure 2, indicating a greater tension of thread therefore a smaller period for the former than for the latter. From the test of the relation between tension and period (see above) one would expect the delays just as we find them in the photographs if the maximum temperature of the muscle was reached just at or slightly before the maximum point of tension.[2]

I conclude therefore that the maximum rate of heat production occurs during the latter half of systole and that the highest temperature of the muscle of the heart is reached during the time of highest internal muscular tension.

4. The total rise of temperature and total heat production during the heart beat. In what was considered the most satisfactory experiment with the Paschen galvanometer the total rise of temperature per heart beat was found to be 0.00132°C. In the present study as already stated this number is 0.00156 and 0.00218 respectively for the two experiments. Assuming no loss of heat by conduction, evaporation, etc., before the rise of temperature is registered by the galvanometer, these figures when converted into gram-calories per gram ventricle are 0.00048, 0.00052 and 0.00073 respectively. Taking the mean of the last two figures, one may express the results of the present study with the Einthoven galvanometer as amounting to 0.00062 calorie of heat liberated per gram of ventricle per beat.

The heat equivalent of Vernon's CO_2 output of surviving turtle's hearts under similar conditions (3) I calculate to be 0.00061 calorie per beat per gram of muscle. It is assumed that the CO_2 arises from the complete oxidation of a definite quantity, the molecular equivalent, of dextrose, which in this case amounts to 1.6×10^{-7} gram.

Assuming also that the heat liberated in my preparations of turtle hearts had its origin in the oxidation of dextrose, the amounts required in the two sets of experiments would be 1.3×10^{-7} and 1.67×10^{-7} gram respectively per gram ventricle per beat.

Turning to actual determinations of sugar consumption by the surviving heart I have at hand only those made upon the mammalian organ at temperatures near 37°C.

In three experiments with the heart of the cat Müller (4) found from 0.0253 to 0.0279 gram of dextrose to disappear per hour. No weights of the hearts or rate of beat are given. But if a weight of 12 grams is assigned to the former and a rate of 120 to the latter a consumption of about 3×10^{-7} gram per gram muscle per beat is indicated. Among the experiments of Locke and Rosenheim (5) there is one in which all the desirable data are given. It is experiment 2, a rabbit heart of 6.25 grams, rate of beat from 156 to 205 per minute. Taking 180 as the mean rate the dextrose consumption per gram of heart per beat appears to be 1.3×10^{-7} gram. Rohde (6) found a consumption of "10 to 40 mg." sugar per gram of cat's heart, indicating a utilization per beat similar to the quantity in Müller's cases, namely 3×10^{-7} gram. Taking the mean of the six determinations of Underhill and Prince (7) on hearts of "well fed" rabbits whose rates of beat "varied from 74 to 144 per minute," the consumption per beat per gram heart is 2.5×10^{-7} gram dextrose.

---

[2] The latency of the mechanical or rather the difference between the latencies of the mechanical and thermal levers, should be taken account of here. But that could only be a question of a hundredth of a second or so and would not affect the result in this case.

Clarke (8) who paid especial attention to the prevention of loss of sugar through the action of bacteria and to the promotion of its utilization by the muscle through the action of the pancreas, obtained an interesting series of variation in dextrose consumption in the consecutive hours of the perfusion. When we are once in a position to consider the total energy output of the heart under its various factors of regulation this data will be of special significance. For the present the mean dextrose utilization in these determinations on hearts (of the dog in this case), when perfused without the pancreas in the path of circulation, may be stated as about 0.175 mgm. per gram of heart per hour. With the pancreas in the path of circulation the mean number is about 0.4 mgm. Assuming the rate to have been on the average 100 beats per minute (it is stated that the hearts beat vigorously) the utilization per beat appears to be about 0.3×10^{-7} and 0.7×10^{-7} gram respectively.

Taken all in all, then, the sugar consumption of the mammalian heart for the most part may be put between 0.3×10^{-7} and 3.0×10^{-7} gram per gram muscle per beat. The quantities of dextrose which are equivalent to the quantities of heat observed to be liberated by the beating chelonian heart lie well within these limits.

BIBLIOGRAPHY

(1) **Snyder**: This Journal, 1917, xliv, 421.
(2) **Rohde**: Zeitschr. f. Physiol. Chemie, 1910, lxviii, 182.
(3) **Vernon**: Journ. Physiol., 1910, xl, 295.
(4) **Müller**: Allg. Zeitschr. f. Physiol., 1904, iii, 282.
(5) **Locke and Rosenheim**: Journ. Physiol., 1907, xxxvi, 205.
(6) **Rohde**: Arch. f. Exper. Path. u. Pharm., 1912, lxviii, 491.
(7) **Underhill and Prince**: Journ. Biol. Chem.. 1914, xvii, 299.
(8) **Clarke**: Journ. Exper. Med., 1916, xxiv, 621.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 NOVEMBER 1, 1918 No. 2

QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

III. Relation of the State of Nutrition of Paramecium to the Rate of Intracellular Oxidation

E. J. LUND

From the Department of Animal Biology, The University of Minnesota

Received for publication July 20, 1918

It is a well known fact, since Rübner's (1) work, that the rate of respiratory metabolism in mammals is markedly increased after ingestion of food, particularly of protein, while during starvation the respiratory metabolism in general decreases (2). Direct experimental data on respiratory metabolism in invertebrates when subjected to starvation is, however, quite meagre (3), (4). The data in this and the following papers are so far as I know the first to appear on the relation of nutrition to respiration in any of the protozoa. The experiments were carried out on a pure line of Paramecium caudatum.

Several investigators (5) have used the degree of susceptibility to cyanide as an indirect method for the purpose of determining the relative "rates of metabolism" of many different organisms under different external conditions and states of nutrition. This method according to these investigators (5, p. 72) depends for its usefulness upon the assumption that cyanide in appropriate concentrations inhibits the metabolic processes in cells, in particular the oxidations. For Paramecium the assumption that potassium cyanide is a specific inhibitor of oxidations is certainly not valid since it has been clearly shown in the previous paper that the oxidations continue practically unchanged often for many hours, depending upon the concentration of the cyanide, until cytolysis takes place.

The relation of the state of nutrition of Paramecium and Didinium to their resistance as measured by their survival time in potassium cyanide solutions has recently been carefully worked out (6). These experiments definitely showed that the resistance as measured by the survival time in the cyanide solutions increased or remained at a high level when food was ingested by Paramecium and Didinium, while starvation of these animals led to a marked decrease in the survival time in cyanide. It is hardly conceivable that acclimation to cyanide as suggested by Child (5, pp. 72–73), could play any rôle in increasing the survival time in cyanide solutions under the conditions of these experiments. The results with the concentrations, n/75-n/85, employed in these experiments must therefore be classed with those of the "direct method." From this we should expect that the starved Paramecia and Didinia would have a *higher* "rate of metabolism" than their fed sisters. What are the facts?

It is very doubtful if the rate of oxidations as measured by carbon dioxide production or oxygen consumption can even approximately be used as a measure of the rate of total metabolism in a cell for it has never been shown that the speed of such processes as hydrolyses and changes in colloidal constitution are correlated to the speed of oxygen consumption or carbon dioxide production. However, if one finds any advantage in speaking loosely of "rate of metabolism," then perhaps one may use the rate of intracellular oxidation as the best available measure in our present state of knowledge. In any case, the safest method for determining the rate of oxidation is by direct measurement of the oxygen consumption or carbon dioxide output.

The following experiments are reported from among a considerable number, all of which agree in showing that the rate of oxygen consumption is much greater in a cell which has recently taken food than in one which is starving, hence the *opposite* to what would be expected if decrease in resistance to strong solutions of potassium cyanide is an index to increase in rate of oxidations.

That the resistance to cyanide or to any other toxic agent is in some way correlated to some condition of the cell is of course not thereby denied. In fact, difference in resistance of different cells under identical external conditions must be due to some kind of difference between the cells.

EXPERIMENTAL

Two methods were adopted for testing the question. For the sake of clearness the actual procedure employed in the first method will be given by describing that used in the experiment, the results of which are given in table 1, and from which the curve, figure 1, was constructed.

Thirty-six bottles of equal volume—137 cc.—were filled with tap water which had previously come into equilibrium with air in respect to temperature and oxygen content. One cubic centimeter of a concentrated suspension of Paramecium gradually transferred to, and washed two times in tap water with the aid of the centrifuge, was added to all but three of the bottles. These blanks served as controls to determine the amount of iodine absorbed by the 1 cc. Paramecia during analysis.

TABLE 1

Suspension of a pure line of Paramecium caudatum left for thirty-six hours in clear native hay infusion, then centrifuged, transferred slowly from native hay infusion to tap water and finally washed in tap water two times. Thirty-six bottles of 137 cc. each, filled with tap water. Three were used as control blanks. To each one of the remaining 33 bottles was added 1 cc. concentrated Paramecium suspension. Dissolved oxygen given in cubic centimeters thiosulfate. Temperature 21 ± 2°C.

BOTTLE NUMBER	BLANKS ANALYZED AT ONCE	1 CC. PARAMECIUM ADDED TO EACH BOTTLE ANALYZED AT END OF											
		Analyzed at once	10 hours	20 hours	30 hours	40 hours	50 hours	60 hours	70 hours	80 hours	90 hours	106 hours	114 hours
	cc. thio.	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*
1	5.6	5.55	4.10	3.60	3.15	2.60	2.10	1.80	1.60	1.0	0.50	0.10	0.05
2	5.7	5.50	4.30	3.50	3.10	2.50	2.10	1.65	1.55	1.02	0.55		
3	5.7	5.50	4.40	3.50	2.90	2.70	2.15	1.75	0.80(?)	1.02			
4		5.45						1.85					
Average....	5.66	5.50	4.26	3.53	3.05	2.60	2.11	1.76	1.31	1.01	0.52	0.10	0.05

Iodine absorbed by 1 cc. Paramecium, 0.16.

Four of the bottles containing 1 cc. Paramecium were analyzed for dissolved oxygen at once to determine the original oxygen content and the amount of the iodine absorbed by the Paramecia which was in this experiment 0.16 cc. At ten-hour intervals after the beginning of the experiment as indicated in table 1, sets of two to four bottles were selected at random from among the remaining and analyzed for their dissolved oxygen. Averages of each set were taken and are given in the table. The variation of room temperature during the experiment which lasted one hundred and fourteen hours or about four and one-half days, did not exceed 2°C. This variation, however, would have introduced noticeable errors in the results if it had not been for the fact that the variations were uniform from day to day and so on the average compensated one another. In other experiments, where necessary, the temperature was kept constant to within 0.2 to 0.3°C. The condition of the Paramecia was carefully watched by means of the binocular to determine if injury or deaths occurred in any of the bottles during the experiment. It often happens that transfer to pure tap water is too sudden or that the centrifuging and mechanical manipulation is too severe. Experiments showing evidence of such unfavorable treatment were discarded and modifications were made in technique so as to eliminate these difficulties in other experiments. It has always been possi-

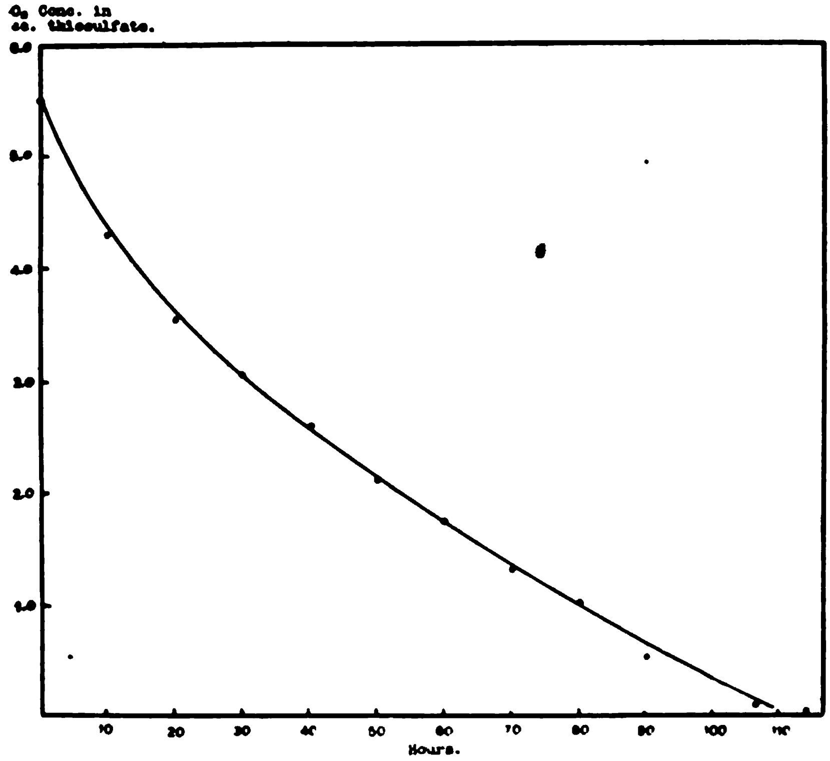


Fig. 1

ble by this means to obtain favorable conditions so that the animals have remained in a perfectly normal and active condition for several days in tap water under conditions of starvation.

The averages of the quantity of dissolved oxygen left at the end of the different time periods are given in cubic centimeters of a thiosulfate solution which was standardized before and after the experiment and found not to have changed. The Paramecia gradually exhausted the supply of dissolved oxygen. The rate at which this supply was used up will be seen from the curve, figure 1. The values on the ordinate represent the average number of cubic centimeters thiosulfate equivalent of dissolved oxygen in table 1, and those on the abscissa the time in hours given in the same table. The rapid fall of the curve during the first twenty hours shows that the rate of oxygen consumption is from two to three times greater at the beginning of the starvation period than later on. The oxygen consumption during the last three days is very nearly directly proportional to the time, since the latter part of the curve is practically a straight line. The close proximity of the points to the curve indicates that the temperature fluctuations that occurred did not, for the purpose of the experiment, appreciably affect the results.

The early period of ten to twenty hours represents the time when the food in the vacuoles and the larger part of the deutoplasmic (food) reserve of the cell becomes exhausted. The protoplasm changes from a gray or dark color in transmitted light to a clear transparent almost colorless condition. A higher initial rate of oxidation immediately after removing from native medium with food to starvation in tap water was also indicated in an experiment in a previous paper (7), figure 1.

During such an experiment the oxygen concentration of course decreases and hence it was at first thought that the above initial high rate of oxidation was due to the relatively high oxygen concentration at the beginning, while the subsequent slower rate was due to the lower concentrations of dissolved oxygen. But this explanation becomes impossible since it has been definitely shown that the rate of oxidations in Paramecium is independent of the concentration of dissolved oxygen (7).

The result of the experiment in table 1 is, however, not in itself conclusive proof that the rapid decrease in rate of oxidation during the first period is due to sudden exhaustion of the major part of the food reserve of the cell for it might be assumed to be due to stimulation of the cell by chemical, osmotic or mechanical environmental changes that necessarily accompany transfer of Paramecium from native hay infusion to tap water. It therefore became desirable to arrange experiments in such a manner that all such objections would be removed by showing that, given two identical lots of Paramecia in the same state of starvation, if one lot is fed and the other left unfed, the fed will consume more oxygen in the same given period of time, under the same conditions, than the unfed. These conditions were fulfilled in the following experiments.

PREPARATION OF THE PARAMECIUM SUSPENSION AND THE METHOD OF FEEDING

Filling the bottles with tap water and preparation of the Paramecium suspensions were carried out according to the method described in a previous paper (8). The animals were placed under varying conditions of starvation in clear native hay infusion, and various dilutions of this clear native hay infusion and tap water. Complete starvation occurred in pure tap water after more or less gradual transfer from the native medium.

The duration or degree of starvation in each experiment is indicated in the descriptions of the tables which follow.

The yeast suspension which was used as food was prepared as follows. One-third of a cake of fresh Red Star or Fleischmann's compressed yeast was suspended in 50 cc. of tap water. A few cubic centimeters of this suspension were boiled in a test tube for two or three minutes. The suspension was shaken to prevent clumping and then quickly cooled. This procedure, of course, kills the yeast and thus prevents consumption of oxygen by the yeast when it is afterward added to the bottles containing Paramecia.

The sample of killed yeast was now washed in tap water two or three times with the aid of the centrifuge in order to remove the extractives which had passed into the water during boiling. This step is important since if the extractives from the boiled yeast along with the yeast itself are added to the water of the bottles which contain the Paramecia, bacteria will sometimes multiply quite rapidly during the experiment and therefore will consume an appreciable amount of the dissolved oxygen. This must be avoided. After carefully adding 1 or 2 cc. of Paramecium suspension to each one of the bottles, about three to five drops of the freshly killed and washed yeast suspension were placed carefully on the *bottom* of each one of the bottles, the Paramecia of which it was desired to feed. All the bottles were immediately stoppered. The Paramecia in the bottles to which killed yeast was added very soon collected on the bottom of the bottles and in an hour or two they were seen to be filled more or less with vacuoles containing yeast The yeast must be carefully placed on the *bottom* of the bottle using a fine long capillary pipette. If such a small quantity of yeast cells is suspended in the water of the bottle the concentration of yeast is too low to enable the Paramecia to "fill up" on the yeast in a short time

TABLE 2

Paramecium starved in one-half native and one-half tap water for forty-eight hours, then transferred to and left in tap water for fourteen hours and finally concentrated with centrifuge and washed twice in tap water just before being used. By count there were approximately 117,000 Paramecium per 1 cc. of the stock suspension. Oxygen given in cubic centimeters of thiosulfate. Temperature 26 ±. 2°C.

BOTTLE	CONTROLS—ANALYZED AT ONCE				ANALYZED AT END OF 9½ HOURS			Remarks
	(1) Blanks	(2) 3 drops yeast added	(3) 1 cc. Paramecium added	(4) 3 drops yeast and 1 cc. Paramecium added	(5) 3 drops yeast added	(6) 1 cc. Paramecium added (starved)	(7) 3 drops yeast and 1 cc. Paramecium added (fed)	
	cc. thio.	*cc, thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	*cc. thio.*	
1	4.07	4.01	4.03	3.93	3.98	3.80	2.98	All active and normal.
2	4.01	4.00	4.03	3.95	3.97	3.70	3.04	
3	4.07	3.89	4.02	3.95	4.03	3.75	2.99	
Average O_2 content..	4.05	3.96	4.02	3.94	3.99	3.75	3.00	
Iodine absorbed.....		0.09	0.03	0.11				
O_2 consumed in 9½ hours.............					+0.03	0.27	0.94	

(9). This is an important condition for the best success of the experiment. More than three to five drops of yeast suspension should not be added for this is an ample quantity of food and also reduces to a minimum the error in the analysis which is due to absorption of iodine by the yeast. The Paramecia were added to the bottles previous to addition of the yeast in order to avoid disturbing the latter. Recent experiments by Miss Wolf in this laboratory have shown that both boiled and living yeast which has been washed in sterile tap water will serve as a satisfactory food for Paramecium for pure line experiments, the medium being sterile tap water. The tap water medium and food can be renewed every day or oftener, if necessary, to prevent bacterial growth; thus, so far as can be judged at present, doing away with the usual variability in the medium and nature of the food when hay infusions are used. Growth and cell division go on at a relatively rapid rate and rhythms in rate of cell division as found by Woodruff (10) are plainly evident. Results of these studies will be published at some later time.

TABLE 2

Paramecia removed from cultures and placed for forty-eight hours in native medium diluted to twice its volume with tap water. They were then centrifuged and washed three times during a gradual transfer to pure tap water over a period of thirty-six hours. Temperature 22.5°C.

BOTTLE	CONTROLS—ANALYZED AT ONCE				ANALYZED AT END OF 9½ HOURS			Remarks
	(1) 2 cc. tap water medium added	(2) 3 drops yeast and 2 cc. tap water medium added	(3) 2 cc. Paramecium added	(4) 2 cc. Paramecium and 3 drops yeast added	(5) 3 drops yeast and 2 cc. tap water medium added	(6) 2 cc. Paramecium added	(7) 2 cc. Paramecium and 3 drops yeast added	
	cc. thio	*cc. thio*	*cc. thio*	*cc. thio.*	*cc. thio*	*cc. thio*	*cc. thio.*	
1	4.60	4.52	4.49	4.38	4.53	4.10	3.79	All living and normal.
2	4.64	4.59	4.35	4.43	4.47	4.07	3.72	
3	4.59	4.57	4.49		4.51	4.14	3.68	
Average O_2 content	4.61	4.56	4.44	4.40	4.50	4.10	3.73	
Iodine absorbed		0.05	0.17	0.21				
O_2 consumed in 9½ hours					−0.06	0.34	0.67	

Several preliminary experiments were carried out in order to test what seemed to be all possible sources of error. Tables 2 and 3 give the results of two of the final experiments all of which agreed in showing a marked increase in oxygen consumption after feeding. The error from the presence of the yeast and the Paramecium are corrected by determining the iodine absorbed by the yeast and Paramecium separately, columns 2 and 3, and collectively, column 4, in each of the tables 2 and 3. By looking at these figures in the tables it is clear that the sum of the amounts of iodine absorbed by the yeast, column 2, and the amount of iodine absorbed by the Paramecia, column 3, are practically equal to the amounts of iodine absorbed by the yeast and Paramecia when placed in the same bottle, column 4. This shows that the corrections are satisfactory and that the control as arranged may be fully depended upon to give values which can be used in determining the actual amounts of oxygen present at the beginning as well as at the end, so far as the errors of the analysis due to the presence of Paramecia and yeast are concerned.

In order to determine if the dead yeast cells which were added to the bottles to be analyzed at the end of the experimental period, column 5 and 7, do consume any oxygen during this period, a control containing only yeast, column 5, was analyzed at the end of the experimental period. The average amount of oxygen consumed by this yeast would of course be the difference between the average oxygen contents of the bottles in column 2 and column 5. This is given at the bottom of column 5 of the tables. It will be seen that this amounts to about 0.05 cc. thiosulfate which is about equal to the largest error in titration with careful work. The amounts of oxygen consumed during the experimental period by the starving Paramecia are given at the bottom of column 6 of the tables, and represent the difference between the average oxygen content at the beginning, column 3, and end of the experiment, column 6. They are respectively, for the two experiments, tables 2 and 3, 0.27 cc. and 0.34 cc. thiosulfate equivalent of oxygen. Since the controls, columns 2 and 5, show that no oxygen has been consumed by the yeast, then it will be evident that the amount of oxygen consumed by the fed Paramecia is equal to the difference between the average oxygen content of the bottles in columns 4 and 7. In table 2 this difference is 0.94 cc. while in table 3 it is 0.67 cc. thiosulfate. The average amount of oxygen consumed in table 2 by the starved Paramecia is 0.27 cc. while that consumed by the fed is 0.94 cc. thiosulfate equivalent, that is, the total oxygen consumed in nine and one-half hours under the same conditions except that of food was between three and four times as much by the fed Paramecia as that by the starved in table 2, and in table 3 it was about twice as much. These differences are very much greater than could be accounted for by errors entering into these experiments. The longer the duration of the starvation the greater within certain limits is the difference in the rates of oxidation of the starved and fed Paramecia. Hence, in order to obtain significant differences in such experiments, the animals must be starved for a sufficient length of time. Several experiments not reported brought out this point very clearly. Furthermore the animals must be washed carefully in tap water to avoid introducing excessive numbers of bacteria. That bacteria did not develop in those bottles containing yeast and so consumed any detectable amount of dissolved oxygen during the above experiments is shown by the equality of the averages in columns 2 and 5 of the tables. To obtain such results it is usually necessary to wash the boiled yeast in sterile tap water as described above, before adding it to the bottles so as not to introduce any dissolved substances which may serve as a source of food for bacteria.

The question arises, was this great increase in oxygen consumption by the fed Paramecia in tables 2 and 3 accompanied by cell multiplication and hence doubling or trebling the amount of living protoplasm during the experimental period of nine or nine and one-half hours? That cell multiplication did not accompany the two- to threefold rise in oxygen consumption after feeding during the above experimental periods becomes evident from the following facts: *a*. In individual pure line cultures in watch glasses, previously starved Paramecia in tap water suddenly fed on boiled yeast usually do not pass through the first division under the conditions of the above experiments until after periods of from twelve to twenty-four hours. The experimental periods were only nine or nine and one-half hours. *b*. Comparative counts of the number of Paramecia in bottles containing the same number of animals at the beginning, the animals of one bottle being fed while those of the other were not, showed that if division did occur during the nine and one-half hours it certainly did not occur to any detectable degree, for the counts of the fed and starved animals at the end of nine and one-half hours were practically the same. Such counts were carried out for the experiments in tables 2 and 3 by a method the details of which cannot be given here. *c*. Examination of the bottles from time to time by the aid of a binocular showed no dividing animals but did show that the cells had eaten the yeast and were in general larger than the starved ones. *From this it must be concluded that the two- to threefold increase in the rate of oxidation accompanied growth of the cell and not cell division in these experiments.* This is furthermore made certain by a consideration of the curve, figure 1 above, which shows that the oxygen consumption was high at the time of removal to tap water and low at the end of twenty to thirty hours. Cell division could of course not account for this result, and since none of the cells died it is evident that the change in the speed of oxidation is in these experiments clearly correlated in some way to growth and starvation processes in the cell.

The results of experiments, tables 2 and 3, similarly support the previous explanation of the early rapid consumption of oxygen shown in the curve of figure 1, namely that this high initial rate was due to the relatively well nourished condition of the cell immediately after removal from native hay infusion as compared to the condition of acute starvation after twenty hours.

Further convincing evidence that the rate of respiratory metabolism in Paramecium after feeding is increased several fold will be given in a following paper on carbon dioxide production. Discussion of the significance of the results will be postponed until all the data have been reported.

The details of experiment and conditions have been given in some detail, if not fully, for the purpose of showing the precautions necessary and the way in which such experiments must be controlled in order to obtain satisfactory data.

SUMMARY

1. When Paramecium is removed from its native hay infusion in a well nourished condition, to a condition of starvation in tap water, the rate of oxidations in the cell decreases simultaneously with the disappearance of the greater amount of the deutoplasmic food reserve of the protoplasm.

2. Feeding boiled yeast to a Paramecium which has been brought into a state of acute starvation by placing it in tap water may increase the speed of the oxidations two or three times its original amount in the acutely starved but otherwise normal active cell. This increase was not accompanied by cell division in these experiments.

BIBLIOGRAPHY

(1) **Rübner**: Gesetze des Energieverbrauchs, 1902.
(2) **Lusk**: The science of nutrition, 1917, 3rd ed.
(3) **Slowtzoff**: Beitr. zur Chem. Physiol. u. Pathol., 1904, iv, 460.
(4) **Brunow**: Zeitschr. f. allg. Physiol., 1911, xii, 215.
(5) **Child**: Senescence and rejuvenescence, Chicago, 1915.
(6) **Lund**: Biol. Bull., 1918, xxxv.
(7) **Lund**: This Journal, 1918, xlv, 365.
(8) **Lund**: This Journal, 1918, xlv, 351.
(9) **Lund**: Journ. Exper. Zoöl., 1914, xvi, 1.
(10) **Woodruff**: Biol. Bull., 1917, xxxiii, 51.

VIII. THE EFFECT OF ADRENALIN UPON THE FATIGUE PRODUCED BY THE INJECTION OF THE FATIGUE PRODUCTS, LACTIC ACID AND ACID POTASSIUM PHOSPHATE

CHARLES M. GRUBER AND OTTO S. KRETSCHMER

From the laboratories of Physiology and Pharmacology and the Henry S. Denison Research Laboratories in the University of Colorado. With the assistance of a grant from the Elizabeth Thompson Science Fund

Received for publication July 24, 1918

Fatigue substances were first demonstrated by Ranke (1) in 1865. He noted the depressing or fatiguing action on skeletal muscle of lactic acid, acid potassium phosphate and carbon dioxide.[1]

The production of lactic acid in contracting muscles and in muscles undergoing death changes was demonstrated by Fletcher and Hopkins (2), Fletcher (3) and others. Lee (4) was the first to record graphically the effect of these substances upon the contracting muscles. He observed that weak solutions perfused through the muscle for a long period of time had the same effect as a strong solution in a shorter time. In both cases a depressing or a fatiguing action was observed. This depressant action of sarcolactic acid, acid potassium phosphate and carbon dioxide was observed in both curarized and non-curarized muscles. A dilute solution transfused through the muscle a short time brought about a temporary increased excitability.

More recently Burridge (5) studied the effects of these substances upon muscles which were excited both directly and indirectly. When he used high concentrations of the acids (0.25 per cent) he found a rapid and complete disappearance of the indirect response, followed shortly by that of the direct. Perfusion of normal saline gave an immediate and apparently complete recovery of the direct response but not of the indirect. In a very short time, however, the response to direct excitation disappeared, regardless of whether or not the perfusion was continued or whether or not the muscle was excited.

---

[1] For a complete discussion of the early literature on fatigue, the reader is referred to an article written by Frederic S. Lee (4).

That adrenalin markedly bettered the height of muscular contraction was demonstrated by Cannon and Nice (6), Gruber (7) and others. Gruber noted that fatigue decreased the threshold irritability of the nerve muscle preparation and that adrenalin increased this decreased irritability. Gruber and Fellows (8) demonstrated that adrenalin counteracted the decreased height of contraction and decreased irritability due to death changes.

In this research we wished to determine whether adrenalin, which possesses the ability to increase the height of contraction and increase the decreased threshold irritability of a fatigued muscle, would counteract the fatigue produced by perfusing moderately strong solutions of fatigue substances through the muscle.

METHOD

The animals, cats, were anaesthetized with ether and then tracheotomized and the administration of ether continued. The tendons of both tibialis anticus muscles were isolated from their insertions and about each was tied a strong ligature. The anterior tibial nerves were isolated and cut and their distal ends fastened in Sherrington shielded electrodes, held securely in place by bringing together and fastening with paper clips the cut edges of the skin.

Both muscles were then prepared for perfusion. The femoral arteries and veins were isolated and cannulae inserted, after which the animal was quickly killed. One limb at a time was used. Perfusion was started in each limb a few minutes before experimentation was begun. The work on the second limb was started eight to fifteen minutes after the animal was killed.

The medium for irrigation was a warm (36.5° to 39°C.) Ringer's solution at a pressure of 85 cm. of water, containing only the oxygen absorbed from the air.

With one loop of cord about the hock and another around the foot just below the fastening of the tendon, the leg was bound to the board. The ligature from the tendon of the muscle, after passing about the pulleys, was fastened to a muscle lever mounted upon a tripod base. The pulleys were arranged so that the muscle pulled in the normal direction. The muscle lever consisted of a piece of light straw 20 cm. in length from the axis to the tip of the parchment paper writing point. The tendon attached 3 cm. from the axis began at the moment of contraction to pull against an initial tension of 60 grams developed in a coiled spring For the first 2.5 cm. excursion of the muscle lever on the drum, this was increased 35 grams. The spring was attached at the same position on the lever as was the muscle. Muscular contraction was, therefore, magnified about 6.66 times.

The strength of the stimulating current was 0.1 ampere in the primary circuit which was derived from a storage battery. The stimulating current in every case was maximal make and break induction shocks obtained from a knife blade key, propelled by a motor. The rate, 84 times per minute in these experiments, was slow enough to produce vasodilatation in the vessels of the stimulated muscle. Both make and break shocks were used to prevent polarization of the nerve.

Merck's lactic acid (which was found to be inactive) and Mallinckrodt's lactic acid (which was found to be dextro-rotary 6.91° at 22.5°C.) were used in $\frac{M}{15}$ solutions in most of these experiments. Sarcolactic acid was also employed in approximately an $\frac{M}{3}$ solution. This was obtained by extracting Liebig's meat extract with ether after precipitation of the proteins with alcohol. The optical rotation before the evaporation of water was dextro-rotary 0.57° at 23°C. Acid potassium phosphate was used in $\frac{M}{9}$ solutions. Adrenalin was used in the form of adrenalin chloride and of crystalline adrenalin, epinephrin, 1:1000 solution. In all cases the fatigue substances and adrenalin were injected at body temperature into the perfusion fluid in the arterial cannula.

RESULTS

Acid potassium phosphate. We found as did Lee and Burridge that acid potassium phosphate markedly depresses muscular contraction after an initial excitation. In figure 1 at *1*, 2 cc. of an $\frac{M}{9}$ solution of acid potassium phosphate were injected into the perfusion fluid. There resulted from this injection an initial increase of about 3 per cent, lasting for six contractions, followed by a marked decrease in the height of contraction of about 78.5 per cent, accompanied by a slight development of contracture. This marked drop in the height of muscular contraction was followed by a slight recovery. At *2*, adrenalin chloride (0.5 cc. of a 1:1000 solution) was injected. The muscular contractions at first

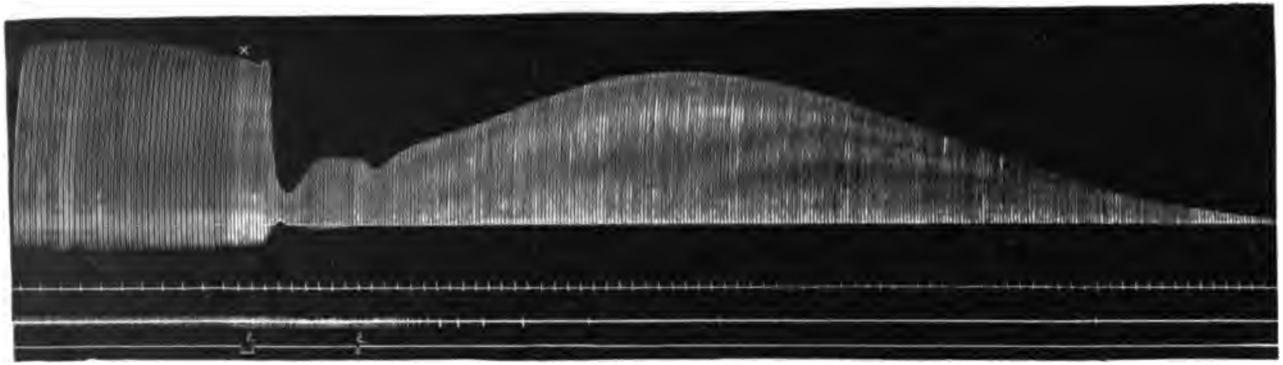


Fig. 1. In this and the following records the upper curve is that of muscular contraction on a slowly revolving drum, the middle line time in 5 seconds, and the lower line the time of the injection. In this figure the rate of perfusion in drops is also recorded. *x*, Slowed drum slightly; *1*, 2 cc. of $\frac{M}{5}$ KH_2PO_4; *2*, adrenalin chloride, 0.5 cc. of a 1:1000 solution. Reduced one-half.

decreased in height. A marked increase followed, the muscle contracting to within 5 per cent of its original height. Adrenalin in this case caused an increase of 128 per cent in the height of muscular contraction. In some muscles after the administration of large, concentrated solutions of acid potassium phosphate, adrenalin neither caused vasoconstriction nor an increase in the height of muscular contraction.

Lactic acid. The results from the inactive lactic acid were not satisfactory. It produced a marked decrease in the height of contraction but adrenalin produced a betterment of only 2 to 4 per cent in some cases and in many it had no bettering effect.

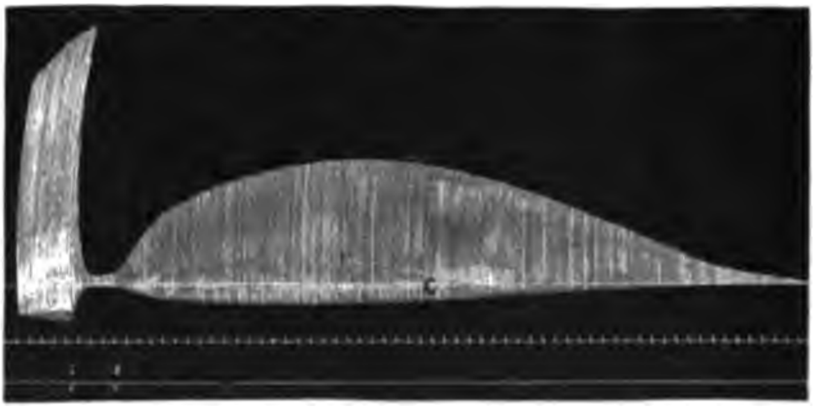


Fig. 2. *1*, 1 cc. $\frac{M}{15}$ Mallinckrodt lactic acid; *2*, epinephrin, 0.75 cc. of a 1:1000 solution. Reduced one-third.

With the use of Mallinckrodt's lactic acid good results were obtained. This acid was dextro-rotary, 6.91° at 22.5°C., which is possibly too high for pure sarcolactic acid. There appeared to be no organic matter or sugar present. In figure 2 at *1*, while the muscle was still developing its treppe 1 cc. of an $\frac{M}{15}$ solution was injected into the perfusion fluid. There resulted an immediate decrease of 95.6 per cent in the height of muscular contraction. At *2*, epinephrin (0.75 cc. of a 1: 1000 solution) was injected from which there resulted immediately a betterment, the maximum being 127 per cent.

Sarcolactic acid. In figure 3 at *1*, while the treppe was still developing 1 cc. of about an $\frac{M}{3}$ solution of sarcolactic acid was injected.[2] There resulted as in figure 2, *1*, a marked drop in the activity of the muscle of 65 per cent with a slight development of contracture. At *2*, epinephrin (1 cc. of a 1:1000 solution) was injected into the perfusion fluid. An increase of 91.7 per cent in muscular activity was the result.

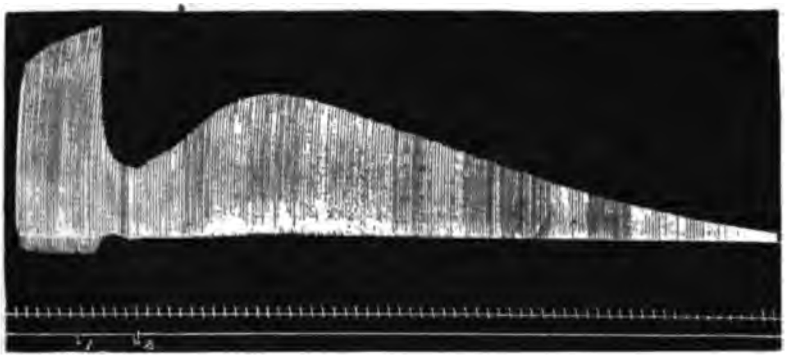


Fig. 3. *1*, sarcolactic acid $\frac{M}{3}$, 1 cc.; *2*, epinephrin, 1 cc. of a 1:1000 solution Reduced one-fifth.

SUMMARY

Adrenalin (0.5 to 1 cc. of a 1:1000 solution) counteracts the induced fatigue produced by the perfusion of fatigue substances such as sarcolactic acid, lactic acid and acid potassium phosphate through the muscle in identically the same way as it does the fatigue produced normally in active muscles.

Adrenalin in some cases has no bettering effect. In these muscles it also has no ability to produce vasoconstriction.

---

[2] On account of the small quantity available the strength of the sarcolactic acid and the optical rotation could not be accurately determined. The optical rotation of this solution when made up to 25 cc. at 23°C. was 0.57° dextro-rotary. This solution of sarcolactic acid, 25 cc., was placed in a beaker and allowed to evaporate to a thick syrupy liquid which amounted to 1 cc. This was then diluted with 3 cc. of water and, if it were pure lactic acid, it would make approximately an $\frac{M}{3}$ solution.

BIBLIOGRAPHY

(1) **Ranke**: Arch. Physiol., 1863, 422; Ibid., 1864, 320; Tetanus, Leipsig, 1865.
(2) **Fletcher and Hopkins**: Journ. Physiol., 1906, xxv, 247.
(3) **Fletcher**: Journ. Physiol., xlvii, 361.
(4) **Lee**: The Harvey Lectures, 1905, 169; This Journal, 1898, ii, 11; Ibid., 1907, xx, 170; Journ. Amer. Med. Assoc., 1906, xlvi, 1492.
(5) **Burridge**: Journ. Physiol., 1910, xli, 285.
(6) **Cannon and Nice**: This Journal, 1913, xxxii, 56.
(7) **Gruber**: This Journal, 1913, xxxii, 221 and 438; Ibid., 1914, xxxiii, 335; Ibid., 1914, xxxiv, 89; Ibid., 1917, xliii, 530.
(8) **Gruber and Fellows**: This Journal, 1918, xlvi, 472.

IX. THE ANTAGONISTIC ACTION OF EPINEPHRIN TO THE FATIGUE PRODUCED BY THE PERFUSION OF ACID SODIUM PHOSPHATE

CHARLES M. GRUBER

From the laboratories in Physiology and Pharmacology and the Henry S. Denison Research Laboratories in the University of Colorado. With the aid of a grant from the Elizabeth Thompson Science Fund

Received for publication July 24, 1918

Burridge (1) noted that acid sodium phosphate had an action upon contracting muscles similar to that produced by the fatigue substances, acid potassium phosphate, lactic acid and carbon dioxide. The muscle showed a marked decrease in the ability to do work after being treated with this acid, the indirect response to electrical stimulation disappearing before the direct response.

Since adrenalin counteracts the fatiguing effects of sarcolactic acid, lactic acid and acid potassium phosphate, readings were made to note if adrenalin would also counteract the fatiguing action of acid sodium phosphate.

METHOD

The method was the same as that described by Gruber and Kretschmer(2). The injections of acid sodium phosphate were always made before the fatigue from excitation could set in. Both make and break shocks were used to avoid polarization and to increase the rate of stimulation.

RESULTS

The results with this acid resemble those obtained by Gruber and Kretschmer upon experimentation with sarcolactic acid, lactic acid and mono-potassium phosphate. A marked drop in the height of contraction usually resulted from the first injection (see fig. 1). Sometimes however, three or more injections were necessary to produce a marked drop in the muscle curve (see fig. 2). In figure 1 at *1*, 1 cc. of a warm $\frac{M}{5}$ acid sodium phosphate solution was injected into the perfusion

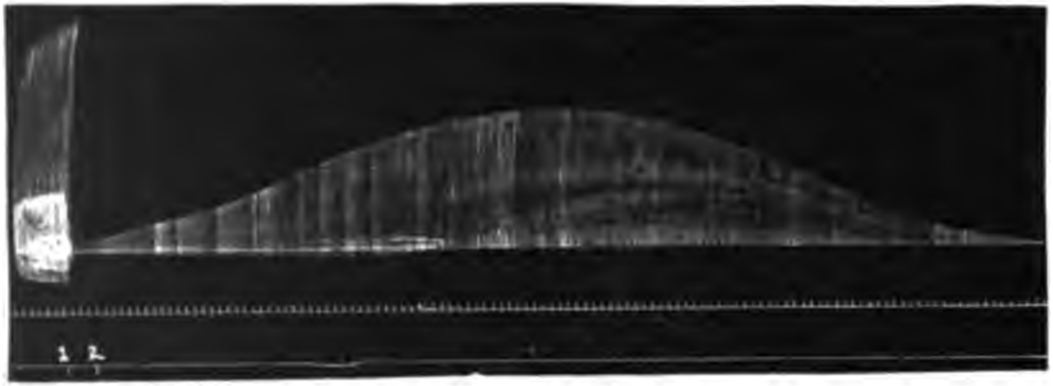


Fig. 1. In this and the following record the upper curve represents muscular contraction, the middle the time in 5 seconds and the lower the point of the injection. Make and break shock contractions. Rate of stimluation was 84 times per minute. *1*, 1 cc. of an $\frac{M}{5}$ NaH_2PO_4; *2*, 1 cc. epinephrin, 1 : 1000 solution. Reduced one-third.

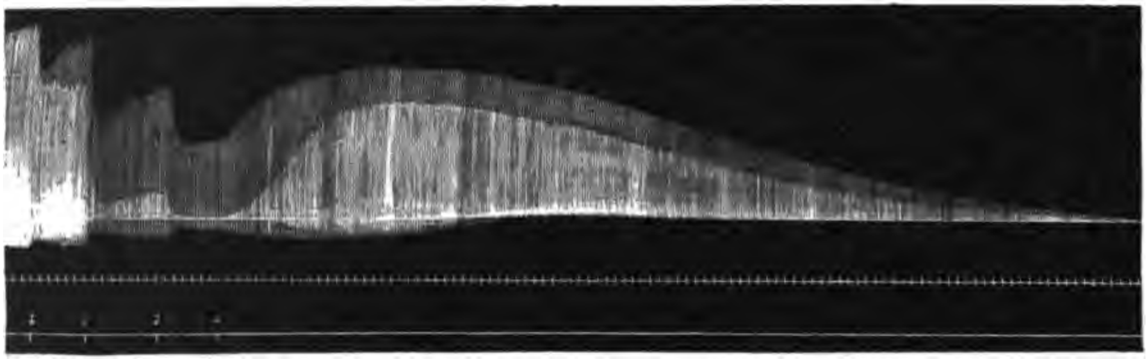


Fig. 2. *1*, 1 cc. $\frac{M}{5}$ NaH$_2$PO$_4$; *2* and *3*, 2 cc. each of $\frac{M}{5}$ NaH$_2$PO$_4$; *4*, epinephrin, 1 cc. of a 1 : 1000 solution. Reduced one-third.

fluid. There resulted a decrease of 97 per cent in the height of muscular contraction. At *2*, epinephrin (1 cc. of a 1 : 1000 solution) was injected, the rate of flow of the perfusion fluid decreased from 60 drops per minute to 1 drop per 5 minutes with an increase in the height of muscular contraction of 104.5 per cent.

In the beginning of the experiment in figure 2 the make shock was maximal but the break shock was super-maximal as shown after the injection of acid sodium phosphate. In this experiment the following injections of $\frac{M}{5}$ acid sodium phosphate solution were made at *1*, 1 cc., at *2*, 2 cc. and at *3*, 2 cc. In each case there was a recovery of the break shock but after the third injection the response to the make shock ceased. It will be noted that after each successive injection the recovery became less steep and smaller in extent. In other cases a slight recovery was noted after the third injection but following the fourth there was no recovery but a gradual decrease in the height of muscular contraction. In these muscles, in which there was this gradual loss as in the muscle from which figure 2 was made, adrenalin markedly increased the height of contraction. In figure 2 at *4*, 1 cc. of epinephrin (1: 1000 solution) was injected; the drops from the venous cannula almost ceased. The height of muscular contraction in response to the break shock increased from 15 to 31 mm. or 106 per cent. The make shocks increased from almost zero to 23 mm. in height.

SUMMARY

Epinephrin counteracts the fatigue produced by the perfusion of acid sodium phosphate as tested by the changes in the height of muscular contraction. In all cases where a marked recovery was obtained, epinephrin caused an almost complete cessation of the passage of the perfusion fluid through the muscle.

BIBLIOGRAPHY

(1) Burridge: Journ. Physiol., 1910, xli, 285.
(2) Gruber and Kretschmer: This Journal, 1918, xlvii, 178.

THE INFLUENCE OF ALKALI ADMINISTRATION ON THE URINARY EXCRETION OF LACTIC ACID, AND THE POSSIBLE SIGNIFICANCE OF THE LATTER IN MAINTAINING NEUTRALITY IN THE BODY

J. J. R. MACLEOD and H. J. KNAPP

From the Physiology Laboratory, Western Reserve School of Medicine, Cleveland, Ohio

Received for publication July 30, 1918

Quite apart from its possible relationship to the diminution of sugar in the blood and urine, the appearance of an excess of lactic acid in the blood following the injection of alkali is of interest in connection with the mechanism by which the acid-base equilibrium is maintained in the body. Because of the great frequency of acidosis as a pathologic condition, this has received much more attention than the opposite condition of alkalosis and the nature of the so-called alkaline reserves which may be called upon to combat any tendency of the H-ion to overstep the normal bounds is thought to be fairly well understood. No attention has, however been paid to the possible nature of the acid reserve available when the H-ion for any reason tends to become less than normal. Under normal conditions of life, there is a tendency to a relative increase in base when the diet contains excess of vegetable food and there may be certain clinical conditions, such as tetany, in which an alkalosis occurs. The acid-base equilibrium under these conditions is probably at first chiefly maintained by neutralization of the excess of base in the plasma by CO_2 and by conversion of NaH_2PO_4 into Na_2HPO_4. Other changes are that more ammonia is converted into urea so that the ratio between urea-N and NH_3-N in the urine falls and some of the alkali combines with protein. Besides these adjustments, the possibility exists that unoxidized acids, such as lactic acid, are used. It was more particularly to investigate this possibility that the present research was undertaken.

It might be assumed that the appearance of an excess of alkali in the organism would be entirely prevented by its combination with carbonic acid. About 0.5 gram of CO_2 are produced by a man of average weight in one minute and this would neutralize about the same amount of base as NaOH, an amount which is many times in excess of what could possibly be absorbed from the intestines in the same period. If the neutralization of excessive base were affected solely in this way, an increase of $NaHCO_3$, and consequently of the carbonate anion HCO_3, would occur in the blood. This follows from the fact that there can be no appreciable change in the ratio of $\frac{1}{20}$ for the molecular equation $\left[\frac{H_2CO_3}{NaHCO_3,}\right]$ provided C_H remains constant. That CO_2 is used in this way (i.e., to form $NaHCO_3$) is shown in results obtained in this laboratory by R. W. Scott, who found, after injecting sodium carbonate solution, that the CO_2-content of the blood increased far beyond the amount that could be accounted for by adding the injected carbonate to the CO_2 already present in the blood. An increase in the carbonate anion has, however, been shown by the work of Hooker, Wilson and Connett (2) and R. W. Scott (3) to cause a stimulation of the respiratory center which is more or less independent of the H-ion concentration of the arterial blood, and such a stimulation of respiration certainly does not occur at any stage during alkali injection. It has recently been shown by the last mentioned worker that marked stimulation of the respiratory center may occur in decerebrate cats when there is an excess of carbonate in the blood, even when C_H of the latter is much below the normal level, indicating quite clearly that the HCO_3 anion itself must be a respiratory excitant.

To prevent accumulation of carbonate in the blood when there is excess of base, therefore, it is theoretically probable that organic acids, such as lactic, are used. We have accordingly determined the amount of this acid contained in the urine of anesthetized animals before and during the intravenous injection of an amount of alkali sufficient to lower C_H of the blood and it has been found that when the base is added in excess of the amount which could be neutralized, the salt of the organic acid accumulates in the blood and appears in the urine. Although a considerable amount of lactic acid was found to appear in the urine during the time of the observation, the quantity excreted was sufficient to account for only a small fraction of the administered alkali. This result raises the question as to whether lactic acid production would occur when the increase of alkalinity was insufficient to affect C_H of the blood appreciably, and if so, whether it could be demonstrated in the urine when excessive amounts of base were absorbed from the intestine.

METHODS

Most of the animals were anesthetized with ether, care being taken to avoid excessive struggling or dyspnea. In one or two cases urethane was used, but not as a rule since this drug may itself lead to lactic acid production. Cannulae were then inserted in the trachea, ureters, femoral artery and femoral vein. Isotonic saline solutions containing varying percentages of Na_2CO_3 were injected through the vein cannula, usually by means of Woodyatt's perfusion apparatus. Samples of about 40 cc. each of blood were removed at intervals from the artery cannula and immediately delivered into tared flasks containing 100 cc. 2 per cent HCl. The flask was then weighed and sufficient of a saturated solution of $HgCl_2$ added to precipitate the protein, the total volume being noted. After removal of the protein and mercury, an aliquot portion of the solution was saturated with $(NH_4)_2SO_4$, further acidified with H_3PO_4 and extracted, without previous evaporation, in either the Lind or the Dunbar apparatus. Our experience shows that it is advisable not to evaporate the blood filtrate prior to extracting with ether, since it is difficult to do this without some loss of lactic acid (due to anhydride formation). After extraction for 48 hours and evaporation of the ether, the residue was immediately oxidized by N/200 permanganate according to the von Fürth-Charnass method.

When urine was used, it was immediately saturated with $(NH_4)_2SO_4$ acidified and extracted with ether, the extract after removal of ether being then oxidized. Ishihara (4) states that it is advisable to precipitate the urine with phosphotungstic acid and then evaporate to small bulk before the ether extraction. Quite apart from the expense and loss of time involved in such a process, the necessity of evaporation to small bulk, even when it is done at reduced pressure, incurs the risk of loss of lactic acid. We find that prolonged ether extraction of fresh urine after saturating with ammonium sulphate removes all of the lactic acid.

We have evidence to show that the bisulphite-combining power of the oxidation distillate is a true measure of the actual lactic acid present when blood is used, but it is doubtful whether this is the case with urine. The ether extract of the latter contains a considerable amount of substance which is oxidized by permanganate so that much more of this has to be added before oxidation is complete than is the case with blood. The distillate also invariably binds a small amount of bisulphite, which is not proportional to the duration of the oxidation process.

This bisulphite-binding substance in the normal urine expressed in terms of N/20 iodine is not large. In a series of dogs' urine, it was as follows: 2.1 cc., 3.3 cc., 1.4. The variations for rabbits' urine, along with the amounts of urine used (in brackets), were: 2.4 (60 cc.); 1.5 cc. (50 cc.); 1.6 (43 cc.); 1.1 (15 cc.); 2.2 cc. (142 cc.); 2.4 (20 cc.); 1.6 cc. (55 cc.); 1.2 (30 cc.); 0.9 cc. (150 cc.); 0.9 (12 cc.). Some of the animals were fed and others starved, which accounts for the variations in the volume of urine but it is clear that the binding substance bears no proportion to the amount of urine. Although the ether was shaken with KOH and distilled after washing and drying over $CaCl_2$, it is probable that a part at least of the binding substance in most instances was derived from this source. Blanks of the reagents alone were not run with sufficient frequency but since conclusions are drawn from the results only when a very pronounced amount of bisulphite-binding substance distilled over, the omission is of no practical consequence.

In view of these facts, we must at present disregard the results on urine unless when the increase in bisulphite-combining power is very distinct, and even then we cannot be certain that lactic acid is alone, or mainly, responsible for the increase. The presumptive evidence is in favor of the oxidizable material being lactic acid, but it would be necessary, before we could be certain, to isolate and identify as the zinc salt and see whether the amount thus recovered agreed with the amount as determined by the permanganate method. We have several times attempted this isolation of zinc lactate from urines showing a large percentage of lactic acid by the oxidation method, but so far without success. Very marked qualitative reactions for lactic acid (Hopkins-Cole) are obtained in the ethereal extracts of such urines but there appears to be something present which interferes with the crystallization of the zinc lactate. Preliminary treatment of the extract with lead carbonate (to remove most of the traces of inorganic salts and of H_3PO_4 which comes over with the ether in the extraction apparatus) and subsequently with charcoal, has not been found to remove satisfactorily all of the interfering substances.

Further details of the purely biochemical aspects of our problem will be published later, but meanwhile we would point out that caution should be exercised in calculating as lactic acid all of the bisulphite-combining substance in the oxidation mixture. We note that this has been done by Dapper (5) and by Ishihara (4).

RESULTS

Lactic acid in the urine during the intravenous injection of sodium carbonate. These observations were made partly on rabbits and partly on dogs. Table 1 gives typical results on two rabbits. In experiment XVI, a total of 8.9 grams Na_2CO_3 was injected at the rate of 1 cc. per minute over a period of about four hours. There was no effect on the respiratory rate until shortly before death, although P_H rose steadily from the normal of 7.4 to 7.8. The urine was excreted in large amount and was removed in 50 to 70 cc. quantities and the lactic acid determined. A steady increase occurred, the total amount excreted during the injection being 0.650 gram. This would suffice to neutralize 4.3 per cent of the injected carbonate.

In experiment XVII, the carbonate solution was twice the strength of that used in XVI, and its injection caused complete apnea after about fifty minutes. The P_H of the blood rose to 7.9 and less urine was excreted than in the previous animal. A total of 0.245 gram lactic acid was excreted, which is sufficient to neutralize less than 2 per cent of the alkali injected.

While the results of these experiments clearly show that lactic acid or something closely related to it, appears in the urine following alkali injection, the quantity is insufficient to neutralize more than a very small fraction of the alkali.[1]

Similar results were obtained by observations on dogs (cf. table 2).

In the first of these (exp. XVIII), the alkali was injected rapidly (about 2.5 per minute) in 2 per cent solution, causing a marked rise in P_H. The urine was freely excreted and in the specimens examined there was a decided increase in lactic acid. The total amount of lactic acid was not determined so that the relationship between this and the amount of injected alkali can not be calculated.

In experiment XIX the alkaline solution was so slowly injected that there was only a slight increase in P_H. The urine was very slowly excreted and contained a total of 0.250 gram of lactic acid or about 5 per cent of the amount required to neutralize the injected alkali. This animal was anesthetized with urethane and is therefore not strictly comparable with the others, in which ether was used.

---

[1] Allowance was not made in calculating the amounts for the bisulphite-binding material in the normal urine and reagents. This is however so small compared with the amounts found after alkali that allowance for it would not materially affect the calculations.

TABLE 1

TIME	RESPIRATORY RATE PER MINUTE	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
				Excreted	Lactic acid	Total amount	
Experiment XVI. Rabbit, weight 2.1 kgm.							
		deg. C.		*cc.*	*per cent*	*grams*	
Started at 5.20	96	39.1		50	0.010	0.005	7.4
6.10	84	38.6	48 cc. 5 per cent solution	50	0.090	0.044	
7.05			40 cc. 5 per cent solution	50	0.120	0.060	7.6
7.55	80	38.0	50 cc 5 per cent solution	70	0.240	0.170	
8.38	78	37.8	40 cc. 5 per cent solution	70	0.300	0.210	7.8
9.30	56	36.8		22	0.760	0.170	
Totals.			8.9 grams Na_2CO_3 (equivalent to 15.1 grams lactic acid)			0.65	
Experiment XVII. Rabbit, weight 3.26 kgm.							
Started at 2.27	96	38		20			7.3
3.20	60	36	50 cc. per cent solution	50	0.220	0.110	
3.55	75		Became apneic. Stopped Na_2CO_3 after 52 cc. injected for 35 minutes. Started again				7.7
4.00					0.340	0.135	
4.30	20–40	36.4	Rabbit apneic. Injection stopped				
		Dead	35 cc. 10 per cent solution	35			7.9
Totals...............			8.5 grams Na_2CO_3 (equivalent to 14.4 grams lactic acid)			0.245	

In experiment XX, even less alkali was given although the P_H of the blood rose to 7.6. Urine excretion was somewhat more rapid and a total of 0.180 gram of lactic acid appeared or about 5.3 per cent of the amount required to neutralize the injected alkali.

In experiment XXI, the same strength of alkaline solution was injected, but more rapidly, with the result that P_H of the blood remained

TABLE 2

TIME	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
			Excreted	Lactic acid	Total amount	
Experiment XVIII. Dog, weight 5.6 kgm. (ether)						
	deg. C.		*cc.*	*per cent*	*grams*	
	Normal 37.1					
2.48	37.6	50 cc. 2 per cent solution				
3.12		38 cc. 2 per cent solution	(2) 50	0.130		7.6
4.05	36.0	50 cc. 2 per cent solution	(3) 20	0.106		
4.10		50 cc. 2 per cent solution				
4.30			(4) 40			7.7
4.45						
4.55		50 cc. 2 per cent solution	(5) 40			
5.15		50 cc. 2 per cent solution				
5.35		50 cc. 2 per cent solution				
5.55		50 cc. 2 per cent solution				
6.00	36.0		60*			
6.20		50 cc. 2 per cent solution				
6.25–26	35.7	45 cc. 2 per cent solution		0.200		7.9
Experiment XIX. Dog, weight 9 kgm. (urethane)						
	Normal 38.5			No urine		7.45
2.20						
3.09	38.3	50 cc. 2 per cent solution				7.5
3.15			11	0.45†	0.050	
3.55	38.5	50 cc. 2 per cent solution				
4.40		50 cc. 2 per cent solution				
5.00						
6.00			19	1.04†	0.200	About 7.6
Totals.........		3 grams (equivalent to 5.1 grams lactic acid.)			0.250 grams (equal to 5 per cent of required amount)	

TABLE 2—*Continued*

TIME	TEMPERATURE (RECTUM)	AMOUNT OF Na_2CO_3 SOLUTION INJECTED	URINE			P_H OF BLOOD
			Excreted	Lactic acid	Total amount	
Experiment XX. Dog, weight 10 kgm. (ether).						
	deg. C.		*cc.*	*per cent*	*grams*	
	38.1					7.45
3.45						
4.20		50 cc. 1 per cent solution				
5.00	37.2	50 cc. 1 per cent solution				
5.05						7.55
5.10			20	0.390	0.0180	
5.50		50 cc. 1 per cent solution				
6.40		50 cc. 1 per cent solution				
6.50			20	0.495	0.100	7.6
Totals.........		2 grams (equivalent to 3.4 grams lactic acid.)			0.180 gram (equal to 53 per cent of required amount)	
Experiment XX. Dog, weight 16.5 kgm. (ether)						
1.05						7.4
1.09‡						
1.29		50 cc. 1 per cent solution				7.4
1.50		50 cc. 1 per cent solution				7.4
2.10		50 cc. 1 per cent solution				7.4
2.31		50 cc. 1 per cent solution				7.3
2.50		50 cc. 1 per cent solution				7.2
3.12		50 cc. 1 per cent solution				7.2
3.31		50 cc. 1 per cent solution				7.3
3.54		50 cc. 1 per cent solution	300	0.130		7.4
Totals . .		4 grams Na_2CO_3 (equivalent to 6.8 grams lactic acid.)			0.390 grams or nearly 6 per cent of required amount	

* Large amount between.

† In both of these the back titration was only 0.2 cc. N/20, so that there was probably more lactic acid.

‡ Ether given.

practically constant but there was marked diuresis. A total of 0.390 gram lactic acid was excreted, corresponding to about 6 per cent of the amount of alkali injected.

In the experiments of the immediately preceding group, the alkali was continuously injected while the urine was being collected. It was thought possible that a larger amount of lactic acid in proportion to the alkali injected might be obtained if the alkali were given within a short period of time at the beginning of the observation and the urine collected for a subsequent period of several hours. The results of two such observations are given in table 3, and from them it appears that contrary to expectation, relatively less of the lactic acid used to neutralize the alkali was excreted in the urine during the observation than when the alkali injections were continuous. In both experiments a marked increase in the percentage of lactic acid was observed in blood which was removed several hours after discontinuing the injections of alkali, and corresponding to this there was a continued excessive amount of lactic acid in the urine. Indeed in one of the experiments the urinary excretion of lactic acid progressively became greater. Had the observation been continued, it is evident that a much larger total amount of acid would have been recovered.

The effect produced by the oral administration of alkali on the lactic acid of the urine. Although the lactic acid excreted in the urine following intravenous injection of alkali is sufficient to neutralize only a small fraction of the administered alkali, the amount thus excreted is so much in excess of that which is apparently present in normal urine that it seemed advisable to see whether any increase would occur when alkali was given by way of the stomach. Since it is uncertain, as has already been pointed out, whether all of the bisulphite-combining material which comes over in the distillate during the oxidation of the ether extract of urine is really lactic acid, one cannot draw conclusions from the results unless decided increases are found to occur. Even in this case we cannot be certain that it is really to lactic acid that the increase is due. Notwithstanding these criticisms, we have thought it advisable to report in condensed form the results which have been obtained.

1. Two observations were conducted on a cat kept on a constant diet of fish and also fed by stomach tube at 9 a.m. daily with a soup made of minced liver, flour and water. On certain days sodium carbonate or bicarbonate was added to the soup. Any effect which the excitement and occasional struggling occasioned by passing the stomach tube might have on lactic acid excretion was largely discounted by the

TABLE 3

TIME	INJECTIONS	URINE			BLOOD		NOTES
		Excreted	Lactic acid, per cent	Lactic acid, total amount	P_H	Lactic acid, per cent	
Experiment XXII. Weigh 13.2 kgm. (ether)							
		cc.		*grams*			
9.40		50.0	0.034	0.0169			Bladder urine removed and cannulae inserted
10.10	4 grams Na_2CO_3 in 200 c. 0.9 per cent NaCl						
10.55							
10.56		116.0	0.057	0.066			Urine alkaline (litmus)
11.20					7.4		Urine slower Blood pressure 130 Temperature 37.8
12.35	5 grams urea injected						Urea injected for diuresis
1.00		36.0	0.130	0.047			
2.45		50.0	0.210	0.105			
2.50						0.187	

Total amount of lactic acid (after subtracting normal), 0.164 gram.

Total alkali injected, 4 grams Na_2CO_3, requiring 6.79 grams lactic acid to neutralize.

The lactic acid excreted sufficed to neutralize 2.1 per cent of administered alkali.

TIME	INJECTIONS	URINE			BLOOD		NOTES
		Excreted	Lactic acid, per cent	Lactic acid, total amount	P_H	Lactic acid, per cent	
Experiment XXV. Weight 13.2 kgm. (ether)							
9.20		80.0					
9.48	2.65 grams Na_2CO_3 injected						No change in blood pressure or respiration by injection
10.17							
10.18							Urine alkaline (litmus)
10.23						0.054	
11.00	5 grams urea injected						Urea for diuresis
12.00		41.5	0.117	0.048			
1.30	5 grams urea injected						Urea for diuresis
3.00	2.5 grams urea injected						Blood pressure 110 mm. Hg. Temperature 38°C.
4.10		51.0	0.094	0.048			
4.12						0.126	

Total amount of lactic acid, 0.096 gram.

Total alkali injected, 2.65 gram Na_2CO_3, requiring 4.5 grams lactic acid to neutralize.

The lactic acid excreted sufficed to neutralize 2.1 per cent of the administered alkali.

procedure being gone through whether or not the soup contained the alkali. Three hours after giving the soup (at 12 noon), the bladder was emptied by catheter and after measuring the specimen of urine so obtained, portions were taken for determination of P_H and ammonia, the remainder being added to the urine which had collected during the previous twenty-four hours in a thoroughly clean metabolism cage. The lactic acid and nitrogen were then determined in the total 24-hour specimen. The cat was fed 100 gram fish immediately after the urine had been collected.

Table 4 gives the result of the two experiments. In cat I, after a preliminary period of eight days during which no alkali was given, there followed one of six days during which 0.5 gram Na_2CO_3 per kg. body weight was mixed with the food daily. This was followed by a third period without alkali.

The carbonate was retained by the stomach except on the second day after the start, when most of the soup was vomited. That the alkali was absorbed and had called into play the neutralizing mechanisms of the body, is indicated by the increase in P_H of the urine and by diminution in the ammonia excretion.

Regarding lactic acid it will be noted that the daily excretion was fairly steady, with the exception of one day, until the alkali period when it rose from an average of about 0.007 gram to 0.012 gram on the first and 0.022 gram on the third days, after which, although the alkali was still being given, it fell back to the normal level. A slight degree of diuresis resulted from the alkali administration, but that this diuresis alone was apparently not responsible for the increase in lactic acid is evidenced by the fact that the actual amount of bisulphite-combining substance in the distillate was increased from a steady average of about 1.7 cc. N/20 for the normal urines to over 4 and 5 cc. on the first and third alkali days, respectively. The increase in supposed lactic acid is, however, very small and the result must be considered as quite inconclusive.

It is interesting to note that on one day in the preliminary normal period (July 24) the lactic acid rose very decidedly and the relative ammonia excretion fell without change in P_H. We have no explanation to offer for this deviation from the otherwise fairly constant daily values.

In the second experiment the procedure was much the same as in the first with the differences that the stomach tube was passed twice daily and the alkali was $NaHCO_3$ instead of Na_2CO_3. It was thought possible that by more prolonged absorption of the alkali from the intestine, more striking results might be secured.

TABLE 4

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
Cat I						
		grams	cc.	gm.		
July 18	50 cc.	0.004	43			
July 19	50 cc.	0.006	56	2.24	4.126	6.1–6.3
July 20	50 cc.	0.006	75	2.27	4.626	6.1–6.3
July 21	50 cc.	0.007	55	1.78	4.118	6.1–6.3
July 23	50 cc.	0.005	80	2.71	4.042	6.1–6.3
July 24	50 cc.	0.020	95	2.50	1.862	6.1–6.3
July 25	50 cc.	0.006	56	2.07	3.661	6.3–6.5
July 26	50 cc.	0.007	90	1.60	5.621	6.3–6.5
July 27	50 cc. + Na_2CO_3	0.012	76	1.85	2.455	8.3+
July 28	50 cc. + Na_2CO_3	Cat vomited				
July 30	50 cc. + Na_2CO_3	0.023	103	2.48	1.360	8.2–8.3
July 31	50 cc + Na_2CO_3	0.007	100	1.72	2.031	8.3+
August 1	50 cc. + Na_2CO_3	0.008	103	0.43	1.976	8.3
August 2	50 cc. + Na_2CO_3	0.005	78	2.47	4.437	8.3
August 3	Normal diet?	0.003	48	Cat vomited and no special determinations made		
August 4	50 cc.	0.012	107	4.3	7.412	8.13
August 6	50 cc.	0.005	80		7.412	6.3–6.5
August 7	100 cc.	0.004	98		4.321	6.3–6.5
August 9	100 cc.	0.007	106		2.919	6.3–6.5
August 10	100 cc.	0.007	70		6.401	6.3–6.5
August 11	100 cc.	0.004	54		2.975	6.3–6.5

Food by stomach, 50 cc. at time, containing 15 grams liver and 10 grams flour.

Fed at 9 a.m. Bladder specimen taken at 12, and after this cat fed in normal fashion approximately 100 grams of fish.

Weight of animal at beginning 2.4 kilos, on August 11, 2.6 kilos.

Na_2CO_3, 0.5 gram per kilo, from July 27 to August 2, inclusively.

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
Cat II. Catheter specimen						
August 7	100 grams	0.005	98		4.32	6.2–6.5
August 9	100 grams	0.007	106	2.65	2.91	6.2–6.5
August 10	100 grams	0.007	70	2.94	6.41	6.3–6.5
August 11	100 grams	0.004	54	2.80	2.97	6.3–6.5
August 14	100 grams plus 2 grams $NaHCO_3$ per kilo	0.014	116	2.90	0.64	8.1–8.3
August 15	100 grams plus 5 grams $NaHCO_3$ per kilo				2.00	8.1–8.3

TABLE 4—*Continued*

DAY	FOOD, ETC.	LACTIC ACID PER DIEM	URINE	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3-N	REACTION P_H
Cat II. Catheter specimen—Continued						
August 16	100 grams plus 5 grams $NaHCO_3$ per kilo	0.004?	62	0.60	0.65	8.3+
August 17*	100 grams plus 5 grams $NaHCO_3$ per kilo	0.050	191	3.80	0.16	8.3+
August 18	100 grams. No alkali	0.007	200	5.80	1.67	7.5–7.7
August 19	100 grams.	0.005	90	2.34	4.73	6.3–6.5

100 gm. or cc. contained 20 gm. of meat and 10 gm. of bread. This was fed by stomach tube in two portions of 50 cc. each, one at 9 a.m., the other at 3 p.m. In the late afternoon the cat was allowed to eat in the normal fashion approximately 100 gm. of fish.

* Cat vomited once on this day.

The P_H of the urine and the relative ammonia excretion again both indicated absorption of the alkali, and the lactic acid excretion on the four days preceding those on which alkali was given averaged 0.006 gram and did not vary greatly. During the first few alkali days there was apparently only a moderate increase in lactic acid (unfortunately the estimation for the second day was lost and there is doubt as to the accuracy of that of the third), but the increase became very pronounced on the fourth, which was also the last day of the period. Diuresis was marked on this day, but it was also present on the succeeding day when, however, the lactic acid had returned to the normal level. This increase was independent of the diuresis, as was shown in the bisulphite-titration figures, which stood steadily at about 1.6 cc. N/20 solution, except in the urine of August 17, when they rose to 9.6 cc. N/20 solution. Unfortunately the cat vomited on this day and although none of the vomit appeared to mix with the urine, yet some may have done so.

An excessive excretion of nitrogen occurred on the last alkali day and on that immediately succeeding,

The results indicate that a substance which is either lactic acid, or something closely related to it chemically, tends to be excreted in greater amount in the urine when alkali is being absorbed from the intestine in sufficient quantity to change the H-ion concentration of this fluid and to lower the relative ammonia excretion. Although the amount of this substance in normal urine is tolerably constant from day to day, yet it does occasionally become very decidedly greater independently of giving alkali (i.e., results of July 24). It is significant however that on the day on which this increase was observed to occur there was evidence of a disturbance in the acid base equilibrium, namely, a fall in the relative ammonia excretion.

OBSERVATIONS ON MAN

These were made on three healthy men living on an average summer diet of approximately the same composition from day to day but without any precise measurement of the actual amounts of its constituents. Care was however exercised to take no irregular amounts either of animal food-stuffs or of fruits and vegetables, since these might disturb the acid base equilibrium. The urine was collected for 24-hour periods with the usual precautions and total nitrogen, ammonia, or total acidity and P_H were determined, as well as lactic acid. After a certain period on the standard diet, sodium bicarbonate was given on several succeeding days and finally the normal diet alone was taken.

The results are shown in table 5. In the observations on K. and Sh., the lactic acid excretion on the normal days varied between 0.014 and 0.029 gram per diem, the amount being independent of the volume of urine excreted. In the case of St., however, the excretion was much greater, viz., between 0.05 and 0.08 gram per diem. We are inclined to believe that the greater values for St. are in part due to more thorough ether extraction. Although this difference in technic occurred between the urines of St. and those of K. and Sh., the extraction was constant for the urines of each experiment so that the results are comparable for any given individual. The more thorough extraction of the urine of St. we attribute to the use of the Dunbar extractor in place of that of Lind.

The amount of lactic acid in normal human urine was found by Ishihari (4) to be about 0.08 gram per liter. Our figures, it will be seen, are somewhat lower. Dapper (5) in a series of pathological urines found daily amounts of lactic acid varying between 0.0516 gram and 0.489 gram, the latter result being obtained in the case of mechanical liver obstruction. Most of the results lay between 0.05 gram and 0.150 gram.

With regard to the influence of alkali administration, it will be observed that the lactic acid excretion was approximately doubled in the urine of K., the increase lasting for at least two days after the alkali administration had ceased. In the case of Sh., the increase was still

TABLE 5

DAY	FOOD, ETC.	URINE	LACTIC ACID, PER DIEM	TOTAL NITROGEN	PER CENT OF NITROGEN AS NH_3N	REACTION P_H
	Subject: K.					
1	Normal diet	920	0.025	10.67	6.68	6.7–6.9
2	Normal diet	930	0.016	10.78	4.96	6.8–6.5
3	Normal diet	1030	0.014	12.33	3.77	6.5–6.7
4	Normal diet	960		10.25	4.28	6.3–6.5
5	$NaHCO_3$, 15 grams per diem	1260	0.039	8.65	1.51	8.1–8.3
6	$NaHCO_3$, 20 grams per diem	1760	0.018	11.59	0.97	8.3+
7	$NaHCO_3$, 25 grams per diem	1300*	0.038	10.69	0.41	8.3+
8	Normal diet	1450	0.033	8.33	5.136	7.1–7.3
9	Normal diet	1070	0.043	11.25	6.08	6.5–6.7
	Subject: Sh.					
1	Normal diet	935	0.029	12.72	4.53	5.5–5.7
2	Normal diet	850	0.023	10.30	3.77	5.5–5.7
3	Normal diet	1100		9.4	5.44	5.5–5.7
4	Normal diet	1250		9.6	5.47	5.5–5.7
5	Normal diet	1100	0.018	11.07	4.06	5.5-5.7
6	$NaHCO_3$, 15 grams per diem	1300	0.022	7.56	2.08	8.1–8.3
7	$NaHCO_3$, 15 grams per diem	1800	0.045	8.07	0.98	8.3+
8	$NaHCO_3$, 15 grams per diem	2300	0.092	8.00	0.83	8.3+
9	Normal diet	1100	0.048	8.10	4.16	5.5–5.7
10	Normal diet	1250	0.093	9.63	5.32	5.5–5.7
	Subject: St.					
					ACIDITY CC. N/10 NaOH REQUIRED TO NEUTRALIZE TOTAL URINE *†	
1	Normal diet	1300	0.068	14.9	247.0	6.1
2	Normal diet	1065	0.080	10.4	303.0	5.9–6.1
3	Normal diet	1050	0.050	8.7	236.2	5.9–6.1
4	Normal diet plus 15 grams $NaHCO_3$	1275	0.092	11.7	92.0	8.0+
5	Normal diet	1210	0.234	13.6	133.1	7.3
6	Normal diet	1315	0.051	17.4	190.7	6.7–6.9
7	Normal diet	1050	0.070	9.2	393.7	6.1–6.3
8	Normal diet	950	0.152	9.12	479.5	5.9–6.1
9	Normal diet	1200	0.103	8.08	462.0	5.8–6.1

* Severe diarrhea.

† The urine was saturated with neutral sodium oxalate and then titrated with N/10 NaOH to the neutral point with phenolphthalein.

more marked, rising from a normal average of 0.025 to 0.091 on the third alkali day; and again the increase continued for at least two days after discontinuance of the alkali.

The observation on St. was conducted in a somewhat different manner from those on K. and Sh. in that the alkali (15 grams $NaHCO_3$) was given on one day only, the urine being collected daily for one week following. On the alkali day a slight increase in the lactic acid excretion occurred and on the following a very decided increase. The excretion then fell to the normal for this individual for two days but rose unaccountably on the next day, when it was accompanied by an unusually high titrable acidity but by no change in P_H of the urine.

In all three observations of this group it will be observed that the H-ion concentration of the urine, the ammonia excretion and the titrable acidity indicate that on the alkali days a sufficient amount of alkali had been absorbed to affect the acid-base equilibrium of the organism.

Taking the results as a whole, the conclusion which we may draw is much the same as that in the case of the observations on cats, namely, that an ether-soluble substance, behaving like lactic acid, becomes decidedly increased in amount in the urine either during or following the administration by mouth of sufficient alkali to disturb the acid-base equilibrium of the body.

OBSERVATIONS ON STARVED AND FED RABBITS

These were undertaken in order to see whether the very decided differences which must exist in the mechanism of acid-base equilibrium in well-fed as compared with starved herbivorous animals would cause any change in the excretion of lactic acid by the urine. Three animals were observed. For a period of five days two of them (rabbits I and II) were starved (water being placed in the cages), and the third fed with abundance of carrots. For a further period of four days the previously starved rabbits (I and II) were abundantly fed. The urine was allowed to collect in bottles containing phosphoric acid placed under the metabolism cages. The urine that collected in twenty-four hours was then saturated with ammonium sulphate, extracted with ether, and lactic acid determined by the usual method. Since the animals were not catheterized, the daily quantities of urine are of course irregular, but it was thought that if the food had any influence on the lactic acid excretion it would be quite evident without such precautions. It was observed that a very large amount of permanganate had to be added to the ether extracts before any permanent pink tinge developed in the oxidation mixture. The amount of bisulphite which combined with the distillate was by no means proportional to the amount of permanganate used, showing clearly that the ethereal extract of rabbits' urine contains a very large quantity of oxidizable material which is not lactic acid.

The amounts of bisulphite-combining material formed, expressed in terms of N/20 solution, were fairly constant for the urines of starved rabbits. This is shown by examination of the following figures, in which the cubic centimeter of N/20 bisulphite combined is given along with the cubic centimeter of urine extracted (in brackets).

1.6 (43 cc.), 1.1 (15 cc.), 2.4 (20 cc.), 1.6 (55 cc.), 1.2 (30 cc.), 0.9 (65 cc.), 0.9 (12 cc.), 0.6 (6 cc.).

TABLE 6

DAY	RABBIT I			RABBIT II			RABBIT III		
	Diet	Urine	Lactic acid	Diet	Urine	Lactic acid	Diet	Urine	Lactic acid
		cc.	mg. grams		cc.	mg. grams		cc.	mg. grams
2	Starved	43	3.3	Starved	15	2.25	Fed	285	9.0
3	Starved	20	4.9	Starved	55	3.26	Fed	290	22.0
4	Starved	30	2.45	Starved	65	1.9	Fed	370	4.5
5	Starved	13	1.85	Starved	6	1.23	Fed	152	1.0
8	Fed	185	10.75	Fed	150	13.1			
9	Fed			Fed	100	6.14			
10	Fed	150	15.54	Fed	85	6.14			
				Fed	95	8.20			

For the fed rabbits, the figures were:

2.2 (150 cc.), 5.1 (150 cc.), 0.9 (150 cc.), 0.5 (152 cc.), 4.0 (150 cc.), 6.4 (156 cc.), 3.0 (100 cc.), 7.6 (150 cc.), 3.0 (85 cc.).

Some of this material may have been derived from the reagents, but certainly not more than 1 cc. N/20 solution, so that the larger amounts distilling over in the case of the urines from fed rabbits must evidently have been derived from something in the urine itself. It is doubtful, however, whether this is lactic acid. In any case the high titrations were always obtained in urines which had been excreted in large volume, although sometimes the volume might be large without any excess of bisulphite-combining material. The results of the experiments, calculated in milligrams of lactic acid excreted in the 24-hour collections of urine, are given in table 6.

In the cases of rabbits I and II there is clearly an increased excretion of what is presumedly lactic acid on the days of liberal feeding as compared with those of starvation. In rabbit III the results are less evident.[2]

In order to eliminate the ambiguity occasioned in these results on account of the great variations in the amounts of urine excreted, another series of observations was undertaken in which the rabbits were all fed an abundance of green food (cabbage) and alkali (bicarbonate) was given on certain days (by sprinkling bicarbonate solution over it). Although the rabbits partook of the alkaline food and the urine volumes were approximately constant from day to day, there was no demonstrable difference in the amount of lactic acid excreted.

CONCLUSIONS

Injection of alkali causes lactic acid, as measured by the von Fürth-Charnass method, to appear in considerable quantity in the urine, and this may occur without any perceptible change in the hydrogen-ion concentration of the blood.

The quantity of lactic acid recoverable during the period of alkali injection is insufficient to account for more than 5 or 6 per cent of the administered alkali.

When the alkali is given in one dose and the urine collected for some hours afterward, the proportion of lactic acid in comparison with the alkali administered is no greater than during continuous alkali administration. The excretion of lactic acid under these conditions is, however, maintained at a high level.

In only a few of the experiments was any decided diminution in respiratory rate observed even when the amounts of alkali injected were sufficient to lower the hydrogen-ion concentration of the blood and urine to a marked degree.

Alkali given by stomach in sufficient amounts to cause a disturbance in the acid-base equilibrium of the body as evidenced by changes in P_H, in the NH_3 excretion and in the titrable acidity of the urine, causes a slight increase in what is presumably lactic acid in the latter fluid. This result was obtained in observations made on two cats and on three men.

---

[2] We are indebted to M. E. Fulk for assistance in several of the experiments on rabbits.

BIBLIOGRAPHY

(1) Macleod and Hoover: This Journal, 1917, xlii, 460.
(2) Hooker, Wilson and Connett: This Journal, 1917, xliii, 351.
(3) Scott: This Journal, 1917, xlii, 196, 1918, xlvii.
(4) Ishihara: Biochem. Zeitschr., 1913, l, 468.
(5) Dapper: Ibid., 1913, li, 398.

VARIATIONS IN IRRITABILITY OF THE REFLEX ARC

IV. Variations in Flexion and Crossed-Extension Thresholds in Experimental Traumatic Shock, with Coincident Blood Pressure Changes

EUGENE L. PORTER

From the Laboratory of Physiology of the University of Pennsylvania

Received for publication August 6, 1918

Diminished reflexes, torpor and lessened sensibility to pain have for long been included as symptoms in typical cases of shock.[1] Just what part the lessened activity of the nervous system plays in producing the completed picture of shock is not yet clear. That it is coincident with other symptoms which are of serious import is beyond doubt. Sir Astley Cooper, quoted by Mansell-Moulin,[2] noted that the moment of transition from intense agony to perfect ease had been known to prove fatal. That diminished activity of the nervous system can be produced in experimental shock is well known to all investigators in this field. Janeway and Ewing,[3] with dogs, are able after sufficient intestinal manipulation to dispense with the anaesthetic. Meltzer[4] reports loss of skin sensitivity in rabbits when the intestines are handled outside the body cavity. Henderson, Prince, and Haggard,[5] produce shock by intestinal manipulation, and as one of the criteria of its presence cite "lessened reflex activity." The phenomenon is not confined to mammals. In Goltz's[6] classical "Klopfversuch," after the frog's abdomen has been struck strongly and repeatedly, the animal passes into a "seemingly dead, stupefied condition." Recovery promptly ensues on cessation of the beating.

---

[1] Compare Fischer's oft-quoted description: Sammlung Klinischer Vortrage, 10 (Chirurgie, 5) 1870, 69.

[2] Mansell-Moullin: International encyclopedia of surgery, N. Y.,1 881, i, 357.

[3] Janeway and Ewing: Ann. Surg., 1914, lix, 158.

[4] Meltzer: Arch. Int. Med., 1908, i, 571.

[5] Henderson, Prince and Haggard: Journ. Amer. Med. Assoc., 1917, lxix, 965.

[6] Goltz: Arch. f. Anat. u. Phys., 1863, xxvi, 1.

Interest in shock at present is largely centered in the redistribution of blood,[7] with all the disastrous consequences of that phenomenon. This feature is doubtless most in immediate need of comprehension and control. It should not be forgotten, however, that serious as the shock effects resulting from a loss of blood from the circulation may be, there exists also a group of primary shock symptoms which "may come on so soon after injury as to be accounted for only as the result of nervous action."[8] These symptoms have been described only from observation on the human patient and on animals with the central nervous system intact, so far as I am aware. In this paper I shall report observations on some primary shock phenomena, as they appear in an animal deprived of the brain. The investigation followed two lines: *a*, quantitative measurement of the increase in thresholds of the flexion and crossed-extension reflexes, which results from the trauma of intestinal manipulation; and *b*, a study of the coincident blood pressure changes and their relation to the high reflex thresholds. The cat was used, made spinal and prepared for a study of the reflexes by methods described in a previous paper.[9] Often, in addition to pithing the brain, the cord has been either pithed or transected, usually not lower than the first dorsal vertebra, sometimes as low as the sixth thoracic. In neither case are the sensory pathways from abdomen to cord interfered with.[10] This was done to eliminate the scratch reflex. In some spinal cats this reflex is very troublesome and if not prevented renders study of the flexion and crossed-extension reflexes impossible. The most certain preventive measure I have found has been to pith a greater or less extent of the cord or to transect it. This operates, presumably, by cutting the reflex arc between the "receptive field"[11] and the hind-legs. The flexion reflex was obtained from the tibialis anticus muscle by stimulation of the posterior tibial nerve with single break induction shocks. Crossed-extension was elicited by stimulation of the same nerve in the same way. The modified Lucas liquid electrode, described elsewhere,[12] was used for stimulation. The threshold for flexion was always determined from a record on the drum. The movement observed in crossed-extension varied in different experiments. Sometimes the leg was held suspended by strings and movement of the entire leg observed; or the femur was immobilized by a clamp which gripped it near the knee, or by a pin passing through a drill hole in it, and extension at knee only observed. Sometimes the patellar tendon was cut and the quadriceps group of muscles caused to write its record on the drum. The threshold for crossed-extension can seldom be determined by any method with as high degree of accuracy as that of flexion. It fatigues more easily and varies more from moment to moment.[13] The changes in threshold produced by intestinal manipulation were so gross, however, as to quite overshadow inaccuracies of threshold determination. The induction coil used was calibrated according to Martin's method,[14] and the thresholds stated in Z-units. Threshold determinations were made as often as once a minute at important points in an experiment; at other times at varying intervals as conditions demanded. The trauma instituted was the one used commonly in producing experimental shock, namely, withdrawing the intestines from the abdominal cavity and manipulating them with the fingers. Sometimes, as a severer measure, they were struck with the handle of a seeker. In many experiments a simultaneous blood pressure record was obtained by a Hürthle membrane manometer connected with the carotid artery. The reflex threshold was usually followed for half an hour or more before manipulation was begun to obtain a basis of comparison, should any change occur.

---

[7] Cannon, Cowell, Fraser and Hooper: Report no. 2, Special Investigation Committee, Medical Research Committee, (Great Britain). Journ. Amer. Med. Assoc., 1918, lxx, 520 and 607.

[8] Cannon: Journ. Amer. Med. Assoc., 1918, lxx, 611.

[9] Porter: This Journal, 1912, xxxi, 141.

[10] Quain: Elements of anatomy, New York, 1909, iii, 139.

[11] Sherrington: The integrative action of the nervous system, New Haven, 1906, 46.

The outstanding features in the results of the investigation are as follows:

1. The flexion reflex threshold may be raised by intestinal manipulation. (Experiment of July 10, 1917, and fig. 1).

---

[12] Porter: This Journal, 1917, xliii, 497. This electrode I have found unfailingly satisfactory. In no experiment in the series of over thirty has there been more than momentary difficulty at the point of stimulation, and the electrode has been in place in some instances six and seven hours. I now use a glass tube 25 mm. long, 5 mm. outside diameter, 3 mm. bore, narrowed in the middle by a very short constriction which reduces the bore to 1.5 mm. Other sizes are needed for very large or very small cats. This form of tube is less likely to result in compression at the point where the nerve leaves it.

[13] Porter: Loc cit.

[14] Martin: The measurement of induction shocks, New York, 1912.

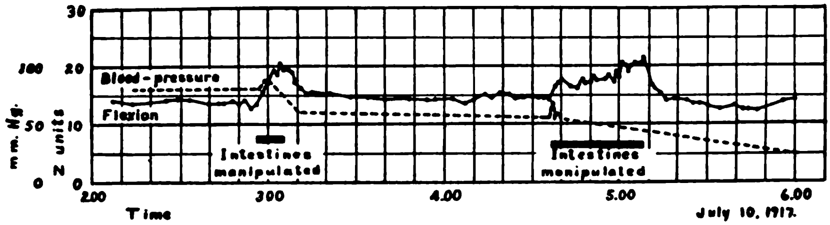


Fig. 1. Increase in the threshold stimulus for the flexion reflex in the spinal cat from manipulation of the intestines. Momentary rise in blood pressure from the same cause. Increase in threshold is apparent in the first reading after manipulation started. Recovery is prompt, eventually complete, and occurs with a lower blood pressure than before manipulation. Ordinates, units of stimulation and mm. Hg.; abscissae, time.

Experiment of July 10, 1917. Medium sized male cat. Brain pithed; cord intact

TIME	FLEXION THRESHOLD	BLOOD PRESSURE	REMARKS
	Z-units	*mm. Hg.*	
2.14	13.9	80	
2.56	13.6	80	
2.56			Manipulation begun
3.00	17.3	90	
3.04	20.6	77	
3.05			Intestines back
3.13	15.5	60	
4.36	14.3	55	
4.37		70	Manipulation begun
4.38	16.8	55	
5.08	21.6	45	
5.08			Intestines back
5.47	12.8	30	

Manipulation twice raised the threshold from a general level of about 14 Z-units to maxima of 20 and 21 Z-units. An appreciation of the amount of this increase is best obtained from the plotted curve (fig. 1). The curve shows marked and unmistakable elevations as a result of the manipulations. The total increase in threshold is six or seven times the maximum changes occurring before manipulation commenced.

2. The threshold begins to rise usually very promptly, a higher level often showing within one minute or less after manipulation has begun. This appears in figures 1, 2 and 5. The maximum threshold

values, however, are often not obtained until manipulation has proceeded for several minutes, as in the experiments of figures 1 and 2. Figure 5 shows an exception. Here in both cases of manipulation the thresholds were higher at the first reading, one minute after manipulation started, than they were at any time later.

3. Upon cessation of the manipulation there is commonly a prompt and more or less complete return to the original threshold (figs. 1 and 5). In the second half of the experiment shown in figure 1 the recovery was complete in eight minutes. It is seldom any quicker.

4. Recovery from the manipulation may be long delayed, as in the experiment of June 15, 1918 (fig. 2).

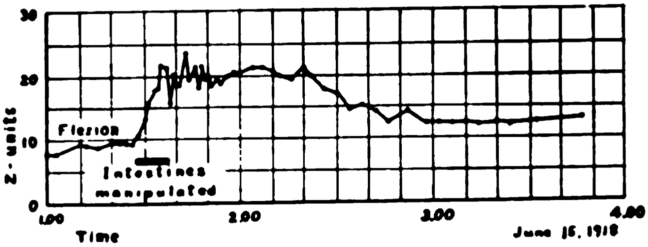


Fig. 2. Increase in the threshold stimulus for the flexion reflex in the spinal cat from manipulation of the intestines. Nearly complete recovery of threshold, but after long delay, as compared with the recovery shown in figure 1. Ordinates, units of stimulation; abscissae, time.

Experiment of June 15, 1918. Small pregnant female cat. Brain pithed; cord pithed to first dorsal vertebra

TIME	FLEXION THRESHOLD	REMARKS
	Z-units	
1.00	7.7	
1.26	9.2	
1.27		Manipulation begun
1.28	10.8	
1.37	21.3	
1.37½		Intestines back
2.10	20.9	
2.58	12.4	
3.32	12.8	

Here it was an hour and twenty minutes after the manipulation ceased before it again reached a low level, which it held steadily. It maintained this level for fifty minutes. This is not a complete recovery, the threshold being some four units above that obtaining before manipulation. A rise of that amount in the same period of time without manipulation would not be expected.[15]

5. The attempt to raise the flexion threshold by intestinal manipulation often fails. Eleven out of twenty-four of my animals failed to show any change which could be attributed to the manipulation. In the following experiment (June 4, 1918; fig. 3), although manipulation was continued for an hour, not only was there no rise in threshold but the lowest readings of the entire experiment occurred while manipulation was in progress.

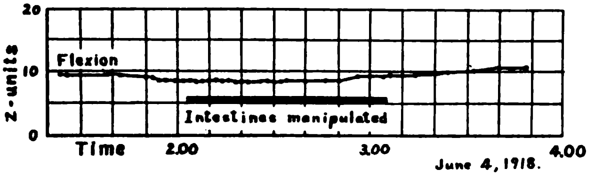


Fig. 3. Failure of the threshold stimulus of the flexion reflex in the spinal cat to be altered by intestinal manipulation lasting over an hour. Ordinates, units of stimulation; abscissae, time.

Experiment of June 4, 1918. Large male cat, much emaciated. Brain pithed; cord cut at first dorsal vertebra

TIME	FLEXION THRESHOLD	REMARKS
	Z-units	
1.24	9.9	
2.02	8.5	
2.03		Manipulation begun
2.20	8.2	
3.05		Intestines back
3.06	9.6	
3.48	10.9	

6. The rise in threshold is not due to a suddenly developed low blood pressure. Low blood pressure suggests itself naturally as a possible cause of high reflex threshold if one follows the analogy of unconsciousness from low blood pressure in syncope. But it is not the explanation here. On the contrary I have always found a rise in blood pressure at the moment intestinal manipulation begins, so that

---

[15] Porter: Loc. cit.

rise in blood pressure and rise in reflex threshold often coincide. This is very clearly the case in the experiment of July 10, 1917 (fig. 1). of which the protocol has already been given (p. 211). Here the rise, in millimeters of mercury, is from 80 to 90 and from 55 to 70, for the two manipulations. In other experiments, and always at the moment manipulation commenced, changes such as these have been observed: 50 to 100, 70 to 120, 30 to 60, 90 to 160, and so on. Figure 4 shows one of the blood pressure records secured during a manipulation. Blood pressure is not maintained at the new level but sinks promptly, often to a lower level, and then gradually diminishes during the remainder of the experiment. (See figs. 1 and 6.) When, therefore, there is a recovery of reflex threshold, it occurs with a blood pressure lower than at any previous time in the experiment. For example, in the experiment of July 10, 1917 (p. 211) at 5.47 the reflex threshold was 12.8 and blood pressure 30 mm. Hg. When the experiment started, at 2.14, reflex threshold was 13.9 and blood pressure 80 mm. Hg. Clearly general blood pressure is not connected with the reflex changes.

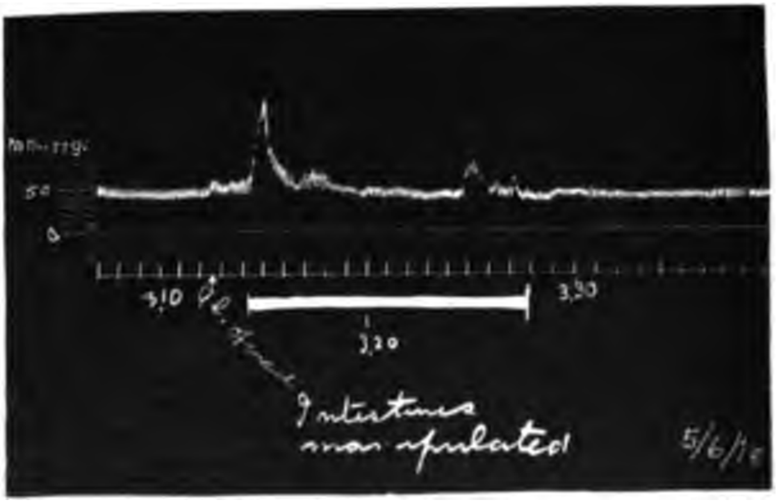


Fig. 4. Momentary rise in blood pressure (from 50 to 150 mm. Hg.) at the beginning of intestinal manipulation in the spinal cat. (Hürthle membrane manometer; calibration at left). Abdomen opened at 3.12½. Intestinal manipulation indicated by the heavy white line.

7. The threshold of the crossed-extension reflex may be raised by intestinal manipulation. (Experiment of May 21, 1918 and fig. 5.)

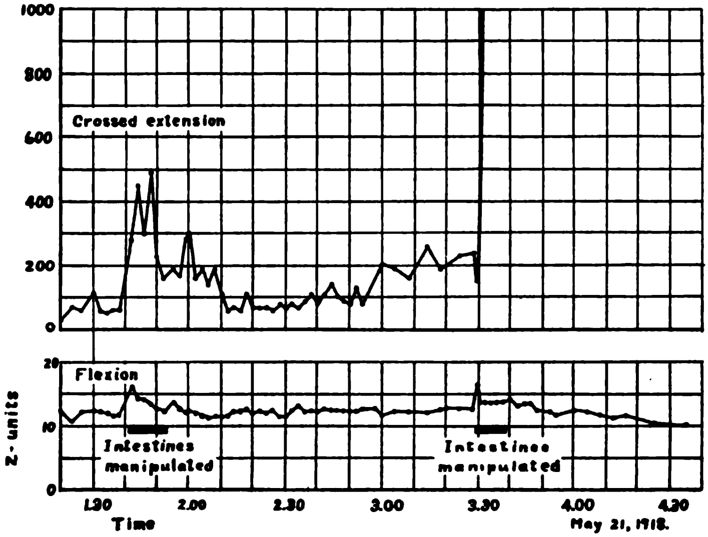


Fig. 5. Increase in the thresholds of both flexion and crossed-extension reflexes in the spinal cat following intestinal manipulation. Highest thresholds for flexion reached immediately after manipulation began (Cf. figs. 1 and 2). Disappearance of crossed-extension after the second manipulation. Ordinates, units of stimulation; abscissae, time.

Experiment of May 21, 1918. Small female cat. Brain pithed, and cord cut at first dorsal vertebra. Crossed-extension observed from movement at knee, femur held in clamp.

TIME	FLEXION THRESHOLD	CROSSED-EXTENSION THRESHOLD	REMARKS
1.20	12.4	32.9	
1.38	11.8	63.0	
1.41			Manipulation begun
1.42	16.1	283	
1.48	13.4	491	
1.52	12.6	163	
1.53			Intestines back
2.12	11.8	63	
3.28	12.8	245	
3.29			Manipulation begun
3.30	13.9	Inelicitable	
3.39			Intestines back
3.40	14.1	Inelicitable	
4.35	10.3	Inelicitable	

The first manipulation raised the threshold to 491 from a general level of less than 100, and the second threw crossed-extension out of action entirely. Obviously the effect of manipulation on crossed-extension is relatively much greater than on flexion. In a few of my animals crossed-extension could not be elicited by single shocks. In others it disappeared before manipulation commenced. In still others it disappeared during or following manipulation, but whether spontaneously or as a result of the trauma could not be told with certainty in all cases. In nine out of the sixteen cases, however, in which a study of crossed-extension was attempted, the reflex was present and could be followed both before and after manipulation.

8. As in the case of flexion, the return of the crossed-extension threshold to approximately the original value may be prompt or delayed. It returned in less than one-half hour in the experiment shown in figure 7; only after an hour and a quarter in the experiment of figure 6. In figure 5 the reflex had returned to its original threshold after the first manipulation, but was unable to withstand the second. Flexion, however, recovered from the second as well or better than from the first. Goltz[16] reports that the production of the "seemingly dead" condition in his frogs, from blows on the abdomen, succeeded better after several repetitions of the experiment on the same animal.

9. I have never found it possible to cause the complete disappearance of the flexion reflex by intestinal manipulation. It is frequently possible to do this in the case of crossed-extension with complete recovery later. (Figs. 6 and 7.) It may however, require long protracted manipulation to bring this about. In the experiment shown in figure 6 manipulation had continued for twenty minutes, and although the threshold was high it was still readable. Four minutes after manipulation had ceased crossed-extension was lost and did not reappear for twenty minutes. In a little over an hour recovery of the original threshold was complete. By disappearance of the reflex I mean that there was no response when the secondary was completely superposed over the primary, giving a stimulation of 1200 Z-units. Stronger stimulation is impractical on account of the violent and confusing flexion response which ensues. The crossed-extension threshold started in this experiment (fig. 6), with the secondary coil 5 cm. from the primary, or 20 cm. from complete superposition. The highest threshold to which I have ever forced flexion as a result of manipulation, is 40 units from an initial threshold of 12 units.

---

[16] Goltz: Loc. cit.

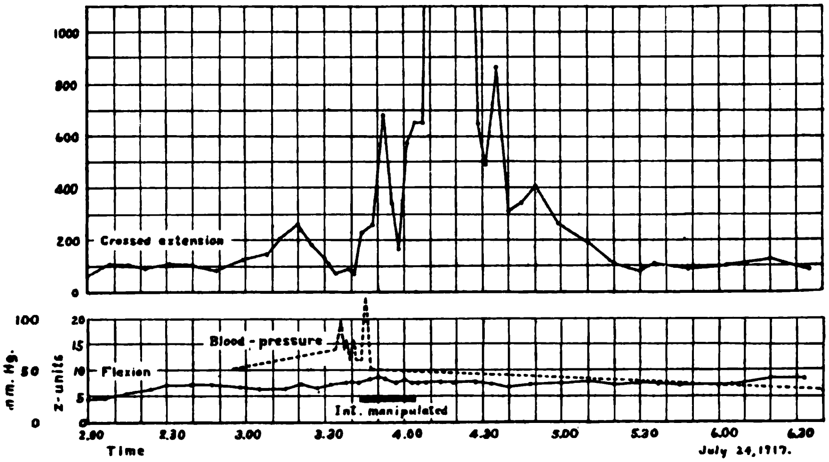


Fig. 6. Increase in threshold of the crossed-extension reflex in the spinal cat from manipulation of the intestines. No effect on flexion threshold. Complete loss of the reflex after manipulation ceased. Return after twenty minutes. Recovery of original threshold one hour later. Brief pronounced rise in blood pressure as manipulation began. Ordinates, units of stimulation and mm. Hg.; abscissae, time.

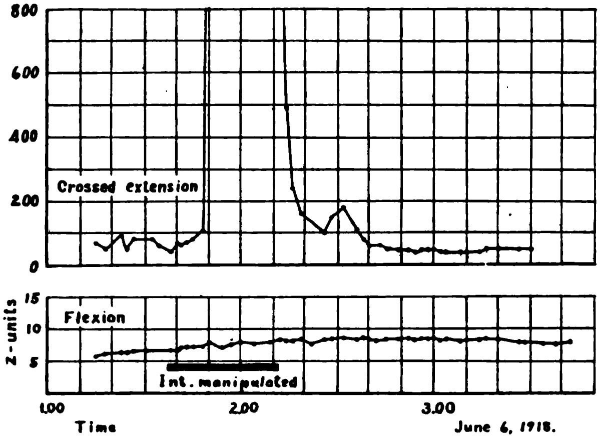


Fig. 7. Loss of crossed-extension reflex after ten minutes of intestinal manipulation. Return within three minutes after manipulation ceased and recovery of original threshold twenty-five minutes later. (Compare the slow recovery in fig. 6.) No effect on flexion threshold. Ordinates, units of stimulation; abscissae, time.

It is evident that the cord, independently of the brain, has its reflex mechanisms seriously disturbed during intestinal manipulation. The mechanism of this alteration is obscure. It may be that it should be classed as inhibition. If filter paper dipped in acid be placed on the frog's abdomen and one sciatic nerve strongly stimulated the foot on the opposite side is inhibited from removing the acid. Or if a sensory nerve causing reflex flexion be stimulated, the motor neurons causing extension are inhibited from action. But such inhibitions are transient; they do not long outlast the stimulus which first aroused them. An impairment of reflex activity lasting more than an hour after manipulation ceased, as in the case of crossed-extension in figure 6, may well belong in a different category. It seems to the writer that the hypothesis of a vasoconstriction within the cord should be tested. Such a vasoconstriction, with anemia, and resultant injury to cord cells would go far toward explaining many of the phenomena described in this paper.

Flexion and crossed-extension are elicited by nocuous stimuli, which, in the intact animal, would result in pain. When, as in the experiment of figure 1, it takes 20 Z-units to elicit the reflex during the manipulation, as compared with 13 units before manipulation, it is probable that 13 units would no longer arouse the same amount of pain in a conscious animal. The results I have described, therefore, may with some probability be regarded as indicating the changes which would be observed in the threshold of pain in the conscious animal during abdominal trauma, if such changes were measurable.

SUMMARY

Manipulation of the intestines in the spinal cat resulted in raising the threshold of the crossed-extension reflex in all cases, and of the flexion reflex in roughly 50 per cent of the animals experimented upon. Increase in threshold may be evident less than a minute after manipulation has commenced. Recovery may be complete or incomplete, prompt or delayed. It is more likely to be prompt and complete in the case of flexion than of crossed-extension. The flexion reflex is never completely lost as a result of the manipulation, crossed-extension often is. A case is described in which this did not occur until some minutes after the manipulation had ceased.

The changes in reflex threshold are not dependent on a decrease in blood pressure; on the contrary, the blood pressure rises momentarily

as manipulation starts, and this may coincide with the rise in reflex threshold. After the momentary rise the blood pressure decreases steadily, as the experiment proceeds, and the original reflex threshold may be recovered under a lower blood pressure than existed before the manipulation.

The suggestion is made, that if pain in the conscious animal could be measured during intestinal manipulation, it is probable that its threshold would show alterations roughly paralleling those of the reflex thresholds described in this paper.

THE VISCOSITY OF URINE

R. BURTON-OPITZ AND ROBERT DINEGAR

From the Physiological Laboratory of Columbia University, at the College of Physicians and Surgeons, New York

Received for publication August 8, 1918

DESCRIPTION OF THE VISCOSIMETER

If different fluids are permitted to traverse a capillary tube under a certain pressure, it will be found that the quantities collected within a given period of time, vary considerably. In explanation of this phenomenon it is generally stated that liquids possess a different internal friction. Thus, if distilled water and blood are employed, it will be found that the former traverses the capillary with much greater ease for the obvious reason that it contains no formed elements nor other material in suspension or solution. But inasmuch as distilled water presents the same differences when subjected to changes in temperature, it is evident that these quantitative differences are not the only cause of this phenomenon. Another factor to be considered in this connection is the viscosity of the liquid which originates in certain qualitative peculiarities of its molecular constituents. It is conceivable that the individual molecules suffer definite physical changes which lead to alterations in their adhesion and cohesion, and in turn give rise to variations in the flow of the liquid. If its relative amounts of water and solids are taken into account, a fluid is usually characterized as "thick" or "thin," while if the character of its flow alone is considered it is said to be either sticky or not sticky. Owing to the very obvious difficulties which any separation of these two factors must necessarily entail the term viscosity is usually regarded as synonymous with internal friction, i.e., it is considered as embracing the quantitative as well as the qualitative peculiarities of a liquid.

In seeking an expression for the viscosity of liquids, reference should be made to the law of Poiseuille (1) in accordance with which the outflow from a capillary tube is determined by the formula:

$$Q = \frac{d^4 h}{l}$$

Q, the quantity collected, is directly proportional to the fourth power of the diameter of the tube and to the height of the pressure, and indirectly proportional to the length of the tube. From these factors the coefficient of the viscosity may be calculated as follows:

$$Q : \frac{d^4 h}{1} = K : \frac{1^4\,1}{1} K = \frac{Q\,1}{d^4 h}$$

The viscosimeter which has been made use of by Burton-Opitz (2) in determining the viscosity of different body-fluids such as the blood, lymph and saliva, consists of a capillary tube through which the liquid to be tested is driven under a definite pressure and in a given period of time. The capillary, C, is adjusted horizontally in a receptacle filled with water. The temperature of the latter is retained at 37°C. by a flame, F, placed underneath the floor of this receptacle. The liquid to be tested is contained in tube R, the caliber of which may be varied in accordance with the quantity at hand. Its size, however, need not be large, because these determinations may be completed with less than 2 cc. of liquid. The end of tube R is connected with a pressure bottle, P, the pressure existing in this entire system being recorded by a monometer, M, upon a slowly revolving kymograph, K_1.

The free end of the capillary tube is placed against a triangular glass slide which moves transversely across its lumen. In this way the liquid escaping from it may be diverted into the weighing glass, W, directly underneath it. A sufficient quantity having been obtained, the lever, S, is moved sideways. This brings a second glass slide in front of the capillary lumen which is intended to divert the liquid into an indifferent receptacle. This arrangement enables us to begin and to cease the collection almost instantaneously.

The movements of the lever, S, are recorded upon kymograph, K_1, underneath the pressure, as well as upon a second kymograph K_2 underneath the record of a Jaquet chronograph. The latter must revolve with a speed sufficient to allow hundredths of seconds to be registered. It will be seen that these lateral deviations of the lever are communicated to an ordinary tambour, D, arranged vertically in front of the contact arm, H, and then by air transmission to tambours adjusted upon the kymographs K_1 and K_2.

This arrangement permits of the determination of the quantity of the liquid which traverses the capillary in a given period of time and under a definite pressure. In addition we know the length and the internal diameter of the capillary and need only determine, as the

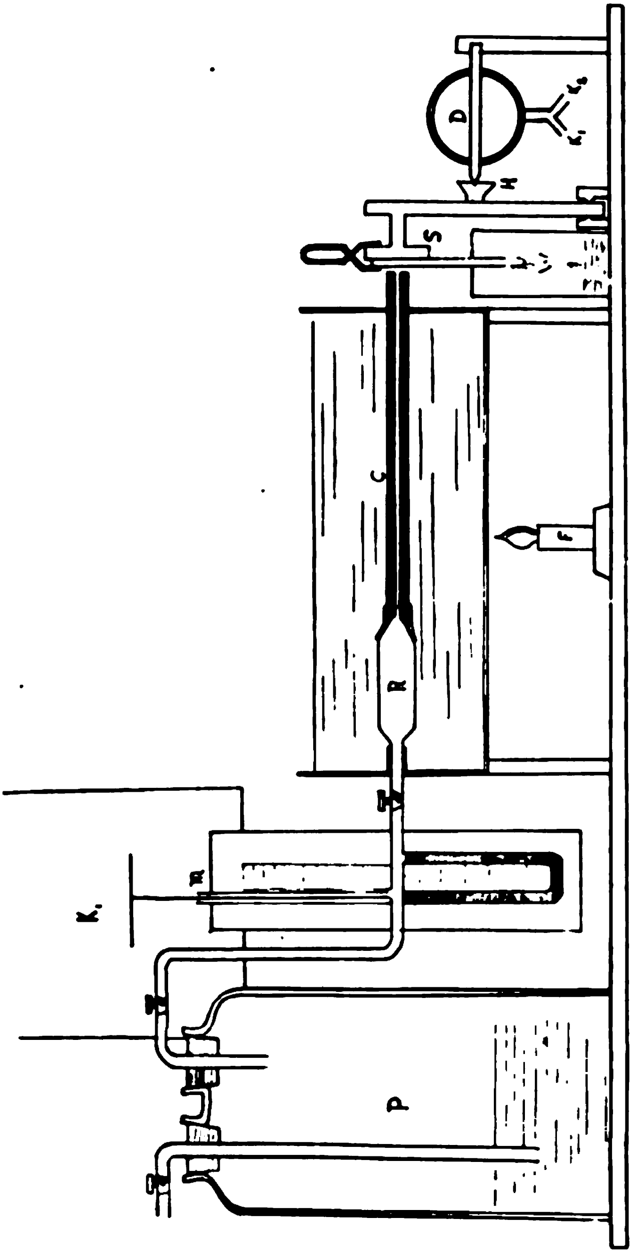


Fig. 1

sixth factor, the specific gravity of the liquid. This last determination is made with small pycnometers either before or after the calibration of the flow. The coefficient of the viscosity obtained with the help of these six factors is then compared with the coefficient of the viscosity of distilled water at 37°C. which, in accordance with the calculations of Poiseuille possesses the value of 4700. This figure is regarded as the standard, so that the viscosity of any other liquid may be expressed in terms of multiples thereof. Thus, if the coefficient of the circulating blood of the dog is 950, its viscosity is about five times as great as that of distilled water at 37°C. Consequently, the viscosity is inversely proportional to the coefficient.

THE VISCOSITY OF URINE

The values pertaining to the viscosity of normal urine are compiled in table 1. These fourteen different samples were obtained from adults upon arising in the morning. It will be seen that the coefficients vary between 3993 and 3239 and that their average value is 3823. Hence, the conclusion may be drawn that normal urine is 1.2 times as viscous as distilled water at 37°C. In this connection brief reference may also be made to the fact that this relationship in the case of the circulating blood of the dog is as 1:5.0, in the case of lymph as 1:1.5, in the case of saliva as 1:1.4 and in the case of bile as 1:1.8.

The aforesaid value corresponds to an average specific gravity of 1.0233 derived from values varying between 1.0165 and 1.0369. In view of the rather limited fluctuation of the viscosity, it seems that this factor serves as an even more reliable means of judging the character of urine than the specific gravity.

The eight determinations contained in table 2 pertain to the viscosity of the urine of normal children between the ages of four and five. In each case the sample was collected in the morning. These coefficients show values ranging between 4236 and 4029 and have given the average value of 4146. This implies that the urine of normal children of this age is only 1.1 as viscous as distilled water at 37°C. The specific gravity of these urines varies between 1.0040 and 1.0163; its average value is 1.0086.

When exposed to a lower temperature urine becomes more viscous, this change being effected more rapidly at higher temperatures than at lower. Consequently urine behaves like water and crystalloid solutions. This is clearly shown by the three experiments compiled in table 3. The differences here recorded for urine at 37° and 5°C.

TABLE 1

The viscosity of normal urine

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
			mgm.	*seconds*	*mm. Hg.*			
1	1......	1.0228	2.5092	37.85	173.4	3836.5	156.5	3914.7
	2......	1.0228	4.3511	64.55	169.4	3993.0		
2	1.......	1.0369	2.9274	45.15	167.4	3833.7	91.8	3787.8
	2.......	1.0369	5.0620	81.15	165.0	3741.9		
3	1.......	1.0238	2.9524	51.64	179.1	3268.0	58.0	3239.0
	2.......	1.0238	3.3788	60.51	176.1	3210.0		
4	1.......	1.0285	3.4506	52.05	173.4	3814.9	4.3	3812.7
	2.......	1.0285	3.0156	45.80	171.7	3810.6		
5	1.......	1.0165	3.3128	49.02	169.7	4020.4	54.2	3993.3
	2.......	1.0165	3.5619	53.75	168.7	3966.2		
6	1.......	1.0169	3.5962	56.50	165.5	3881.6	86.6	3838.3
	2......	1.0169	3.3301	54.60	162.2	3795.0		
7	1......	1.0223	3.0214	48.55	169.5	3686.0	58.1	3773.1
	2......	1.0223	3.4861	59.09	158.2	3744.1		
8	1......	1.0277	3.2181	54.60	154.3	3814.6	7.1	3818.1
	2......	1.0277	2.9038	49.55	153.1	3821.7		
9	1......	1.0185	3.0851	52.35	150.3	39.510	25.9	3938.0
	2......	1.0185	3.0852	53.30	148.6	3925.1		
10	1......	1.0260	2.7280	43.45	165.7	3790.4	107.4	3844.1
	2	1.0260	3.3000	51.80	163.5	3897.8		
11	1......	1.0200	2.8335	46.05	160.2	3864.7	97.6	3913.5
	2	1.0200	3.2444	52 08	158.2	3962.3		
12	1 . . .	1 0235	2 2481	36 42	155 1	3990.9	34.6	3973.6
	2 ..	1 0235	3.7512	61.85	153 8	3956.3		
13	1......	1 0202	3.7429	65.27	150.5	3833.1	17.0	3841.6
	2......	1.0202	2.7440	47.80	150 0	3850.1		
14	1	1 0231	3 6758	56 41	169 4	3858 1	23.9	3846.1
	2	1 0231	3 1653	49 52	167 2	3834 2		
Average .		1 0233						3823.8

TABLE 2

The viscosity of urine of children

EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
		mgm.	seconds	mm. Hg.			
1 { 1........	1.0064	3.1416	54.80	140.6	4158.3	15.9	4150.3
2........	1.0064	2.6453	47.62	137.4	4142.4		
2 { 1........	1.0040	4.1266	71.20	140.2	4226.0	16.5	4234.2
2........	1.0040	3.0782	53.63	138.3	4242.5		
3 { 1........	1.0103	3.7820	66.60	138.5	4165.0	40.7	4144.6
2........	1.0103	2.6336	47.91	135.4	4124.3		
4 { 1........	1.0081	2.7531	50.10	132.8	4212.6	48.4	4236.8
2........	1.0081	2.5409	46.95	129.3	4261.0		
5 { 1........	1.0068	3.3268	60.70	138.5	4033.7	68.7	4068.0
2........	1.0068	2.6418	47.81	137.3	4102.4		
6 { 1........	1.0098	3.3525	60.50	136.7	4120.3	19.4	4130.0
2........	1.0098	2.7916	51.81	132.3	4139.7		
7 { 1........	1.0073	3.5858	64.20	136.4	4172.0	7.6	4175.8
2........	1.0073	2.8900	52.81	133.4	4179.6		
8 { 1........	1.0163	3.2724	61.88	133.9	4006.5	45.6	4029.3
2........	1.0163	1.7743	53.50	130.9	4052.1		
Average..	1.0086						4146.1

TABLE 3

The viscosity of urine at different temperatures

EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
		mgm.	seconds	mm. Hg.		
1 { At 5°C...........	1.0242	2.0454	72.25	114.4	2479.6	1566.6
At 20°C...........	1.0217	3.2388	73.85	153.1	2877.6	
At 37°C...........	1.0161	4.3407	73.82	146.8	4046.2	
2 { At 5°C...........	1.0304	2.5113	81.31	133.1	2312.9	1670.6
At 20°C...........	1.0280	3.0732	72.34	150.5	2818.3	
At 37°C...........	1.0238	3.8681	69.35	142.4	3983.5	
3 { At 5°C...........	1.0324	1.7630	58.12	136.0	2217.3	1637.3
At 20°C...........	1.0263	1.8628	57.71	134.9	2383.8	
At 37°C...........	1.0261	3.2311	61.74	135.8	3854.6	

amount to about 1600 points, corresponding to a viscosity almost twice as great as normal. It is noted, however, that the increase between 37° and 20°C. is very much greater than that between 20° and 5°C. so that a curved line is obtained, the concavity of which is turned upwards. In perfect harmony with these changes it is observed that the specific gravity increases on cooling; the greatest difference here obtained amounts to as much as 8 points.

The effect of water drinking is clearly betrayed by the experiments contained in table 4. In each case three glasses of water were taken very shortly after the completion of the determination of the normal viscosity. A second determination was then made after an interval of from thirty to ninety minutes. While all three experiments show a very decided diminution in the viscosity of the urine, the first has given a difference of more than 300 points, thus establishing a relationship of 1 : 1.1 as against the normal relationship of 1 : 1.2. The specific gravity shows an equally decisive decrease from 1.0224 to 1.0068. Experiments 2 and 3 have yielded somewhat slighter differences for the reason that the second samples of urine were collected after a briefer interval of time, namely after sixty and thirty minutes respectively.

TABLE 4

The viscosity of the urine after water drinking

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	seconds	mm. Hg.		
1	Normal	1.0224	3.9566	67.72	153.7	3816.0	314.1
	After water	1.0068	3.4914	56.92	151.4	4130.1	
2	Normal	1.0184	3.4320	61.21	143.0	3951.3	248.8
	After water	1.0063	3.5544	60.92	141.4	4200.1	
3	Normal	1.0202	3.7429	65.27	150.5	3833.1	138.9
	After water	1.0068	3.1683	55.71	146.3	3972.0	

Table 5 embraces three experiments showing that the deposition of the urates increases the viscosity in a considerable measure. The greatest difference was recorded in experiment 3 and amounted to 1670 points. In other words, while the sample of normal urine was only 1.1 as viscous as distilled water at 37°C., the urates increased this value to 2.0. The specific gravity pursued in this case a course parallel to the viscosity.

Ammoniacal urine shows a tendency to become more viscous while its specific gravity remains practically unaltered (table 6), but care must be taken not to add a larger amount of ammonia than is absolutely necessary to deposit the phosphates. The greatest difference here obtained amounts to only 85 points and cannot therefore be said to be of special significance.

TABLE 5

The viscosity of urine on deposition of urates

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm.* Hg.		
1	Normal	1.0261	3.2311	61.74	135.8	3854.6	1637.3
	Urates	1.0324	1.7630	58.12	136.0	2217.3	
2	Normal	1.0161	4.3407	73.82	146.8	4046.2	1566.6
	Urates	1.0242	2.0454	72.25	114.4	2479.6	
3	Normal	1.0239	3.8681	68.35	142.4	3983.5	1670.6
	Urates	1.0304	2.5113	81.31	133.1	2312.9	

TABLE 6

The viscosity of ammoniacal urine (phosphates)

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE
			mgm.	*seconds*	*mm.* Hg.		
1	Normal	1.0261	3.2311	61.74	135.8	3854.6	16.3
	Phosphates	1.0259	3.1272	67.14	121.4	3838.3	
2	Normal	1.0235	3.6244	77.51	116.6	4021.2	73.8
	Phosphates	1.0235	3.4263	72.83	119.5	3947.4	
3	Normal	1.0224	3.9566	67.72	153.7	3816.0	85.9
	Phosphates	1.0235	2.3101	44.81	138.3	3730.1	

Table 7 contains the determinations of the viscosity and specific gravity of ten samples of albuminous urine. Two tests were made in each case; the average coefficient derived from these being given in the last column of the table. Accurate quantitative data regarding the percentage of albumin present in these urines not being available, the figures in the third column have been obtained by contrasting these different samples with urine which on boiling, became practically solid. The latter was assumed to contain 1.0 per cent of albumin.

The average coefficient of the viscosity amounts to 3829 and the average specific gravity to 1.0197. It will be noted, however, that

TABLE 7

The viscosity of albuminous urine

EXPERIMENT NUMBER	SPECIFIC GRAVITY	PER CENT OF ALBUMIN	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
			mgm.	*seconds*	*mm. Hg.*			
1 {1 . .	1.0109	0.7	3.7589	63.30	147.4	4090.4	39.7	4110.2
2 . . .	1.0109	0.7	2.8278	47.80	145.4	4130.1		
2 {1	1.0137	0.7	3.1888	51.00	153.4	4126.4	79.1	4165.9
2 . . .	1.0137	0.7	2.9171	46.20	152.0	4205.5		
3 {1	1.0200	0.5	2.7908	47.70	149.5	3930.1	25.2	3942.7
2 . . .	1.0200	0.5	3.1620	54.10	148.4	3955.3		
4 {1 . .	1.0164	0.6	2.7343	56.51	120.4	4057.9	37.4	4039.2
2 .	1.0164	0.6	2.8671	61.20	118.0	4020.5		
5 {1 .	1.0089	0.5	2.4728	53.40	120.4	3912.8	57.6	3941.6
2 . . .	1.0089	0.5	2.4772	53.25	119.2	3970.4		
6 {1 . . .	1.0331	1.0	2.1617	60.60	116.8	3024.6	26.9	3038.0
2 . . .	1.0331	1.0	1.8162	51.24	113.4	3051.5		
7 {1 . . .	1.0227	0.9	2.0807	46.95	115.2	3860.7	67.7	3894.5
2 . .	1.0227		1.9965	44.35	115.0	3928.4		
8 {1 .	1.0219	0.9	2.1824	46.10	125.4	3823.4	57.0	3851.9
2 .	1.0219	0.9	2.0698	43.22	124.8	3880.4		
9 {1 . . .	1.0238	0.9	2.8931	49.97	169.5	3441.8	84.3	3483·9
2 . . .	1.0238	0.9	2.7644	46.91	168.4	3526.1		
10 {1 .	1.0255	0.8	3.5766	61.00	151.3	3878.8	94.3	3831.6
2 .	1.0255	0.8	2.5454	44.70	150.6	3784.5		
Average	1019.7							3829.9

these determinations fall into two groups of five each, the first of which presents the average value of 4039 and the second the average value of 3619. The average specific gravity amounts to 1.0139 and 1.0254 respectively. This difference in the results appears to be dependent upon the fact that the samples of urine employed for experiments 6 to 10 contain casts. Obviously, therefore, the ordinary glomerular nephritis gives rise to a urine of low viscosity because the ratio here found is as 1: 1.1. The involvement of the tubules, on the other hand, increases the viscosity considerably so that the ratio becomes as 1: 1.3· The urine used for experiment 6 was obtained from a case of eclampsia. Its specific gravity of 1.033 corresponds to the coefficient 3038. Consequently, this urine is almost 1.6 times as viscous as distilled water at

TABLE 8

The viscosity of diabetic urine

EXPERIMENT NUMBER	SPECIFIC GRAVITY	CONTENT IN SUGAR	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE COEFFICIENT
		per cent	*mgm.*	*seconds*	*mm.* Hg.			
1 { 1.....	1.0072	2.5	2.3442	47.70	124.9	4009.9	94.6	4037.2
2.....	1.0072	2.5	2.0968	42.05	123.8	4104.5		
2 { 1.....	1.0217	2.5	2.7776	42.15	165.3	4004.8	19.5	4014.5
2.....	1.0217	2.5	3.2883	50.02	164.1	4024.3		
3 { 1.....	1.0247	2.7	3.7180	79.05	125.4	3756.8	35.8	3738.9
2.....	1.0247	2.7	2.8376	61.80	123.6	3721.0		
4 { 1.....	1.0213	2.5	2.4084	44.75	134.9	4009.2	17.2	4017.8
2.....	1.0213	2.5	2.2441	41.52	134.9	4026.4		
5 { 1.....	1.0302	2.7	2.1501	43.20	136.4	3635.1	14.0	3628.1
2.....	1.0302	2.7	2.1056	43.20	134.1	3621.1		
6 { 1.....	1.0235	2.7	1.1568	35.65	84.8	3837.3	70.7	3872.6
2.....	1.0235	2.7	1.3402	39.38	83.4	3908.0		
7 { 1.....	1.0231	2.5	1.3083	36.95	72.4	3929.2	92.1	3975.2
2.....	1.0231	2.5	1.0517	36.90	71.3	4021.3		
8 { 1.....	1.0210	2.0	2.8190	45.95	158.2	3895.5	67.6	3929.3
2.....	1.0210	2.0	2.8586	46.15	157.0	3963.1		
9 { 1.....	1.0389	3.2	2.0739	46.75	125.8	3483.4	53.0	3456.4
2.....	1.0389	3.2	2.1091	48.90	124.2	3430.4		
10 { 1.....	1.0221	2.7	3.0693	55.20	148.9	3750.1	39.5	3730.3
2.....	1.0221	2.7	2.8801	52.81	147.6	3710.6		
Average	1.0233							3840.0

37°C. as against the normal ratio of 1: 1.2. These changes are also clearly betrayed by the specific gravity, but it appears that this factor is a much more uncertain means of determining the character of urine than the viscosity.

The ten experiments compiled in table 8 have been made with urine collected from patients suffering from diabetes mellitus. Excepting the first two cases in which a dicided polyuria was present, the coefficients of the viscosity do not differ from normal. Experiments 1 and 2, however, show that polyuria decreases the viscosity. The greatest difference here recorded amounts to about 200 points and corresponds to a ratio of 1.1 as against the normal ratio of 1.2.

BIBLIOGRAPHY

(1) POISEUILLE: Ann. d. chim. et d. phys., Paris, 1843.
(2) BURTON-OPITZ: Pflüger's Arch., 1900, lxxxii, 447; This Journal, 1917, xlv, 25; Biol. Bull., current number.

STUDIES ON EXPERIMENTAL SURGICAL SHOCK

F. C. MANN

From the Mayo Clinic, Rochester, Minnesota

Received for publication September 7, 1918

I. GENERAL CONSIDERATIONS OF EXPERIMENTAL SURGICAL SHOCK

In the consideration of the entire field of surgical shock from the experimental point of view, two features must be emphasized: *a*, that the condition termed shock by the surgeon is undoubtedly owing to a large number of causes; and *b*, that experimentally it is very difficult to reproduce the environment and all the phenomena which the surgeon calls shock.

The present series of studies has been made in order to give experimental data of as many conditions as possible which the surgeon might diagnose as shock. The data are based on a very large number of experiments performed since the first studies were undertaken in 1912, up to the present time. For purposes of investigation I have found it of practical value to classify the various conditions into two groups. In the first group are those cases in which the cardinal signs of the condition, such as decreased consciousness, decreased blood pressure, shallow, gasping respiration, subnormal temperature, etc., develop and supervene for some time after the exciting cause. In the second group are those cases in which a severe or fatal issue follows immediately or very closely on the action of the exciting agent.

Clinically, the first group would include most of the cases which the present-day surgeon diagnoses as shock; the chief of these would be the shock following operation. A condition presenting the clinical signs of shock and which is supposed to present a physiologico-pathologic condition similar to that in the cases in the first group, may be produced by a variety of methods. The most important of these are deep anesthesia, hemorrhage, exposure of the abdominal viscera, the use of excessive heat or cold, the isolation of large vascular areas as the partial occlusion of the vena cava, the production of cerebral anemia and the injection of drugs, peptones and oils.

It may be seen from the foregoing that but few of the methods used to produce the shock condition can be compared to the manner in which shock occurs clinically. Most of the methods aim, primarily, at a reduction of blood pressure. It is definitely known that some of them, and probably most of them, produce a decrease in the volume of circulating fluid. While the studies following these various methods of producing an experimental condition having the signs of shock have been of inestimable value in that they have added to the knowledge of the physiology of low blood pressure, it should be noted that they explain in only a limited number of cases what the possible etiologic factors in shock could be.

The second group, clinically, includes most of the classical cases of shock given in the literature of the subject. It was in reference to this type of case that the term shock was first applied, namely, when sudden death occurs or alarming symptoms develop immediately following an accident or operative procedure in which no definite cause of death is found. It is shock of this type, particularly that occurring in the operating room, that has strongly impressed the surgeon with the idea that the nervous system is essentially and primarily at fault in the production of shock.

The environment reproducing the condition included in the second group is hard to obtain experimentally. Very little experimental work has therefore been done on the investigation of sudden death associated with accidental trauma or trauma occurring in the operating room. The crushing of joints in decerebrated animals has occasionally produced death, and the sudden trauma to the whole thigh of an anesthetised animal has also been fatal.[1] In general, experimental work has not reduplicated the clinical observations in regard to the class of cases included in this group.

Practically all investigators of the shock problem have undertaken the investigation with the idea of proving or disproving some particular theory regarding it. Since the condition termed shock is probably due to a large number of factors, our researches must necessarily have to do with the many possible causes of obscure death. As further data are presented it seems that each of the theories developed to explain the condition of shock contains an element of truth, and that typical cases diagnosed as shock could be found to illustrate each theory. In the present studies no attempt has been made to correlate the data

---

[1] Personal communication from Dr. E. D. Brown.

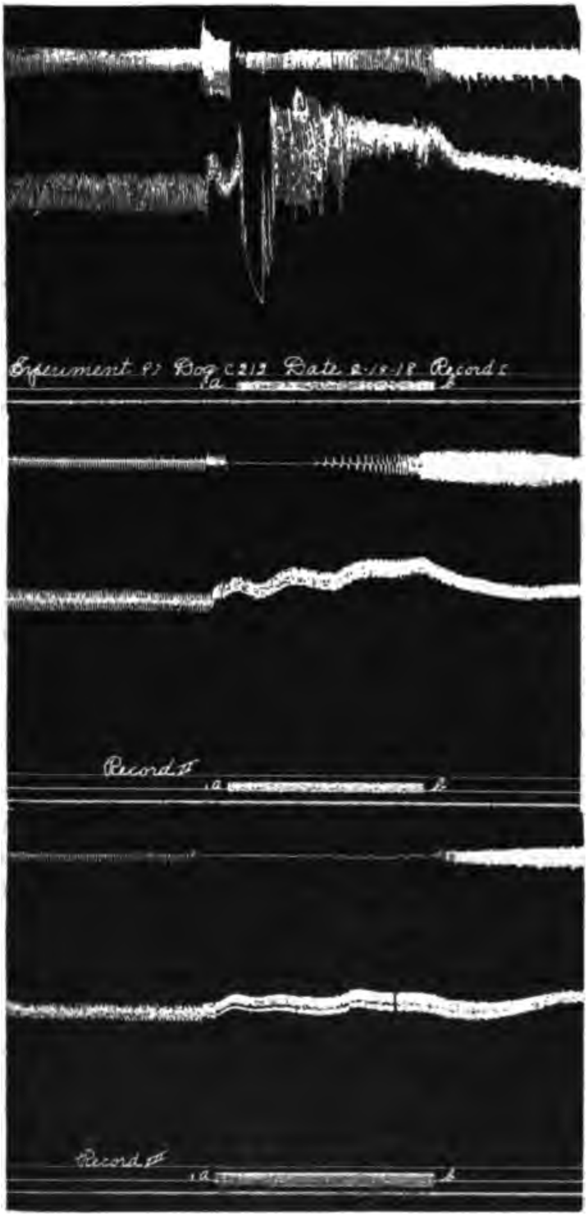


Fig. 1. Photograph of kymograph record showing the effect of different tensions of ether on the inhibitory reflex of respiration. In each record signal *A* marks the time of disconnecting the ether and signal *B* the stimulation of the right superior laryngeal nerve for two minutes. Both vagi are intact. In record 1 ether tension was 40 (uncorrected) and blood pressure was 150. Note that there was but slight inhibition of respiration. The heart was inhibited. Ether tension was gradually increased and in record 2 blood pressure was still 150 but respiration was decreased in rate and amplitude. Note the increase in the time respiration was inhibited. Record 3 shows the effect of another increase in the tension of ether. Respiration is inhibited for a much longer period.

with any of the current theories but all experiments have been devised with the view to determine some facts in regard to the many phases of the shock problem.

I am fully aware that all of these studies are not directly associated with the shock problem. However, until a comprehensive and scientific definition of shock based on known facts is made, it seems best to classify all data bearing on the causes of sudden death, the causes of low blood pressure and the phenomena of shock under the general term surgical shock. A fact that is universally true of experimental work and especially true in regard to shock should also be emphasized, namely, that direct clinical application of the experimental data should be cautiously made.

II. ETHER ANESTHESIA IN RELATION TO SURGICAL SHOCK

The anesthetic is of the greatest importance to the clinician in a consideration of post-operative shock. It is of even greater importance to the experimental investigator who would attempt to determine the causative factors in this consideraton.

All our experiments have been performed under ether anesthesia and therefore all conclusions drawn must be in regard to that anesthetic only. There were two reasons for the use of ether. First, it is the anesthetic used in the greater number of operations and therefore the results obtained will have the greatest clinical application; second, it is the anesthetic which can be most easily employed in experimental work.

In the beginning of our work on the relation of ether anesthesia to shock, we attempted to establish some definite data in regard to the activity of the various organs, nerve centers and reflexes under different tensions of ether. Boothby, by means of the Connell anesthetometer, showed that in man the anesthesia became complete at a definite tension of ether. It is reasonable to suppose that animals would react to ether in a like manner and that a standardization of ether anesthesia in reference to physiologic research would be accomplished. Two great difficulties were, however, encountered. First, the Connell anesthetometers which we personally used and as tested by the Waller gas balance, were found to have too large an error for standardization, and second, the only ether to be obtained at present is not pure enough for accurate work. These two sources of error, which we have not as yet been able to obviate, have necessitated the use of approximations only in relation to ether tensions. The errors in these approximations are on an average probably no greater than 15 per cent. However, the data have justified several conclusions that have been of great value in our work on shock. These conclusions all have reference to ether anesthesia, as administered by an anesthetometer.

1. In general, the physiologic phenomena due to ether are remarkably constant at the same range of tensions in different dogs.

2. Under a low tension of ether, just sufficient to produce narcosis, the blood pressure remains normal for a period of at least twelve hours.

3. The various respiratory and vascular reflexes disappear at different ranges of ether tension.

4. The signs of shock can be readily produced by high tensions of ether.

It is possible for practical purposes to divide ether tensions in relation to their anesthetic action into four groups. In the first group are included the tensions that are too low to produce anesthesia. The second group includes those under which the animal is reduced to a state of surgical anesthesia but which are not high enough to depress any of the vital processes; it is impossible to kill an animal by any tension in this group, if time is not allowed to become a factor. The third group includes the tensions that produce depression of the vital processes and under which death may occur. Any tension higher than those included in this third group is incompatible with life. The range of tensions included in these various groups is approximately constant for the various animals. Of course, individual variations occur but these are usually within the limit of the error of the mechanism.

The employment of approximate ether tensions has been productive of several important findings. By the use of low tensions it can be demonstrated that an animal may be maintained in as normal a condition as it is possible to keep a narcotized animal, for many hours. This is of great value since it is thus possible to maintain a safe degree of anesthesia without its being affected by any physiologic condition of the animal or by operative procedures. It is also of great value, especially in shock experiments, to be able to administer a tension of ether which is definitely known to be too low to maintain surgical anesthesia. It is thus possible, without completely withdrawing the ether, to know when the animal has reached shock.

The respiratory and vascular reflexes undoubtedly vary under different tensions of ether but are fairly constant under the same tensions. Some reflexes are very sensitive to ether and cannot be elicited except under low tension. Other reflexes will persist as long as the involved nerve cells functionate. In the study of any particular reflex it is of as much importance to keep the anesthetic constant as to keep the strength and rate of stimulus constant. This is impossible under most of the methods of anesthesia.

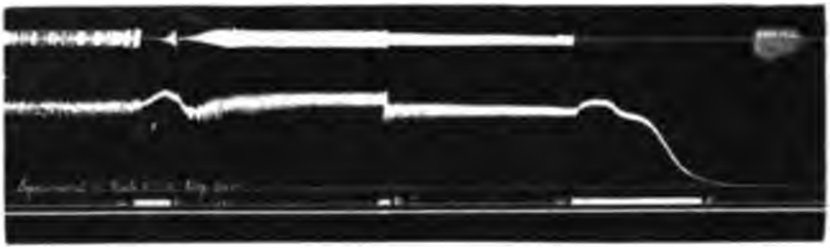


Fig. 2. Kymograph record of respiration and blood pressure. The animal at first was under a moderate ether tension (42 uncorrected). The right vagus was sectioned; the left vagus was intact. At signal *A* ether was disconnected and at signal *B* the central end of the right vagus was stimulated until respiratory movements which were inhibited at first, returned. This occupied 40 seconds. At signal *C* ether was again administered. Signal *D* marks an interval of fifteen minutes during which a high ether tension was administered (64 uncorrected). At signal *A'* ether was again disconnected and at *B'* the central end of the right vagus was stimulated for three minutes. Respiration was inhibited and after an initial rise blood pressure fell to zero.

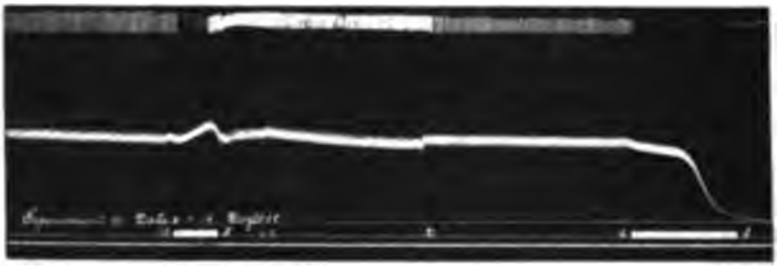


Fig. 3. Photograph of kymograph record of respiration and blood pressure. The procedure and results in this experiment were almost identical with those shown in figure 2. The minor differences are: the initial blood pressure was 120 and the uncorrected ether tension was 40; signal *D* represents a period of fourteen minutes.

A review of protocols dealing with studies of experimental shock shows that in many instances the investigator was undoubtedly studying a condition of deep etherization and not of shock. This is probably one of the most important reasons why so much of the experimental data on shock is contradictory. The relation of deep etherization to the signs of shock cannot be too strongly emphasized. By the manipulations of the various tensions of ether it is possible to parallel the various stages and signs of shock. Under high tensions the blood pressure is decreased and all the other symptoms follow. However, it is quite possible to saturate an animal with ether at a tension just slightly lower than that necessary to abolish the eye reflex and to produce an obtundity of the reflexes without decreasing the blood pressure to a shock level. It is possible to obtain such results with a certainty only when the ether is administered mechanically and is not dependent in any way on the respiratory efforts of the animal.

III. REFLEX INHIBITION OF RESPIRATION AS A CAUSE OF SUDDEN DEATH DURING OPERATION

In a study of the vascular and respiratory reflexes under various tensions of ether (1) it was found that all the respiratory reflexes, except that which produces inhibition of respiratory movements, disappeared before the respiratory center failed. The excitatory respiratory reflexes disappear under a relatively high tension of ether. On the contrary, instead of a depression of the inhibitory reflex being caused by ether, a relative increase at least is quite common. Thus it was possible under deep etherization, in some instances, actually to kill an animal by prolonged stimulation of the nerve fibers that inhibit respiration. It was believed that this phenomenon might have some bearing on the shock problem; a more complete study of it was therefore made.

The idea that death could actually be produced by the action of a nerve reflex has been very prevalent with clinicians but has very little clinical or experimental evidence to support it. For this reason our experiments are important even if all the factors involved have not been determined and even if it is not possible at the present time to make a definite clinical application of the data.

The experiments were performed on dogs. The animals were etherized in a closed cabinet, intubated and the anesthesia usually maintained with a Connell (2) anesthetometer. In some experiments a modified McGrath (3) method of anesthesia was employed in order to determine if the method of administering ether were a factor in the results obtained. Respiration and carotid blood pressure were recorded (mercury, and in some experiments, membrane manometer). Ether was always discontinued during the periods of stimulation. The

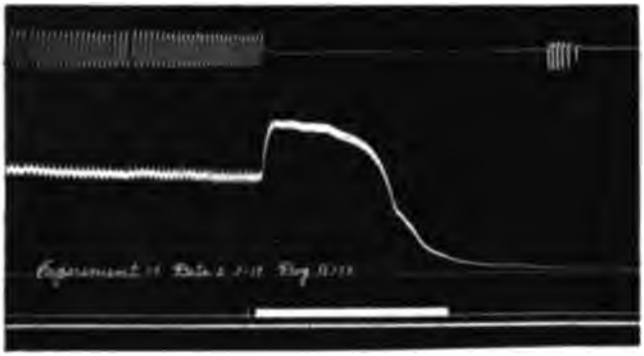


Fig. 4. Kymograph record of blood pressure and respiration. The animal was under a tension of ether which just abolished the eye reflex. Respiration was fifteen per minute and blood pressure was 105. Both vagi were sectioned. Ether was disconnected just previous to the period of stimulation. The central end of the left vagus was stimulated for two minutes and forty-five seconds. Death followed and was associated with inhibition of respiration and an initial rise in blood pressure.

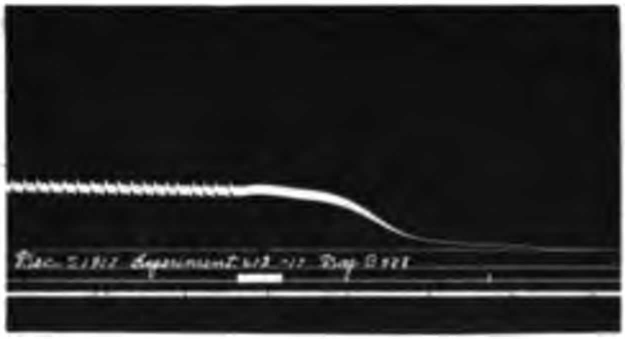


Fig. 5. Photograph of kymograph record showing sudden death following stimulation of the central end of the right vagus for thirty seconds under a high ether tension. Both vagi were sectioned. The animal had been under ether for several hours and under the same tension (38 uncorrected) for one hour. The blood pressure and respiration had been practically constant for the half hour preceding the stimulation. This record proves that death was certainly associated with an active inhibition of respiration.

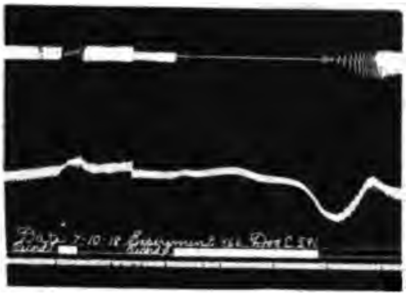


Fig. 6. Photograph of kymograph record showing recovery after a long period of inhibition. McGrath's method of anesthesia was used. The right vagus was sectioned, the left vagus was intact. Stimulation of the central end of right vagus under light ether produced partial inhibition of respiration for less than fifteen seconds (record 1). The ether tension was then increased until the eye reflex had just disappeared. Stimulation of the central end of the right vagus now inhibited respiration completely for the entire period of stimulation. When death appeared inevitable stimulation was stopped. Respiratory movements immediately occurred and the animal soon recovered (record 2). This record proves that at least in some instances death following inhibition of respiration under deep ether is an active process throughout.

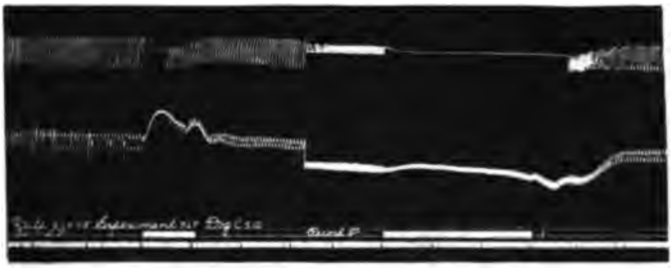


Fig. 7. Photograph of kymograph record showing increase in the inhibitory reflex of respiration under high ether tension. Both vagi were sectioned. In record 6 blood pressure was 120 and ether tension 30 (uncorrected). Stimulation of the central end of the right vagus for one minute produced inhibition of respiration for about half that period. The ether tension was then increased to 70 (uncorrected). When the eye reflex had just disappeared the vagus was again stimulated (record 7). Blood pressure had decreased to 90. The stimulation was maintained for two minutes and respiration was completely inhibited. It remained inhibited after the cessation of stimulation and blood pressure continued to fall. Insufflation was then started and in a short time recovery took place. This record shows that lack of air appears to be the important factor.

nerves were stimulated electrically in the usual manner, care being taken to keep the stimulus as near constant as possible for each experiment. In many experiments shield electrodes were used. The stimulus was always maximum in strength and rate.

From the results of the stimulation of the nerves in a large series of animals it was found that only two that were easily exposed could be relied on consistently to produce reflex inhibition of respiration. Stimulation of other nerves, such as the brachial plexus, occasionally caused a cessation of respiratory movements but only the superior laryngeal and the central ends of the vagi produced constant results. Without doubt, however, the same results may be obtained by the stimulation of other nerves when they are found to inhibit respiration, particularly those nerves containing sensory fibers supplying the upper respiratory tract.

The stimulation of either the superior laryngeal or the central end of a vagus nerve under light surgical anesthesia usually inhibits respiration for a short period. As the stimulation is continued, however, respiratory movements soon return, due either to a decrease in the reflex, or what is more probable, to an increase in the chemical stimulation of the center. It is rarely possible, under light surgical anesthesia, to inhibit respiration by the stimulation of these nerves for a long enough period to jeopardize the life of the animal. In our experiments, excluding those in which reflex inhibition of the heart was associated with the inhibition of respiration, blood pressure was never permanently lowered to a shock pressure. In a few experiments, however, there was marked depression of the blood pressure owing probably to stimulation of the depressor nerve, and complete recovery did not take place. Under ether tensions considerably less than those necessary to produce surgical narcosis, reflex inhibition of respiration might become dangerous provided the circulation was not capable of compensating for a long period of asphyxia. As the ether tension is increased the length of time that the respiratory movements are inhibited by the stimulation of those nerves is prolonged. Finally, in a large number of animals under deep etherization, the respiration fails to return; the blood pressure quickly falls and death ensues. Quite frequently death can be produced by reflex inhibition of respiration under an ether tension which will just abolish the eye reflex. A fatal result did not always occur in our experiments but the respiration, with very few exceptions, was inhibited for a much longer time under deep etherization than under light anesthesia; the opposite was rarely true.

In the earlier experiments the normal blood pressure was usually decreased as much as one-half before respiration could be inhibited long enough to produce death. However, it was found that by cautiously increasing the ether tension many animals could be killed while the blood pressure was practically normal. It was determined that such results are not dependent on the method of anesthesia or the height of blood pressure, and that they are obtained with sectioned and intact vagi.

The mechanism by means of which death is produced, associated with an inhibitory reflex of respiration under deep etherization, is not clearly defined. In our experiments death occurred only when the nerves that inhibit respiration were stimulated, and it was never produced under deep etherization when respiratory movements were maintained. Death will not take place while insufflation or artificial respiration is maintained. The processes producing death seem to have been as follows:

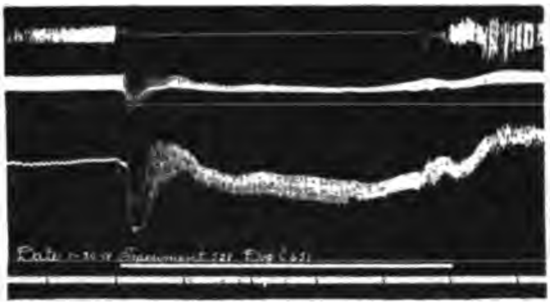


Fig. 8. Photograph of kymograph record showing a long period of inhibition of respiration associated with reflex inhibition of the heart following stimulation of the right superior laryngeal nerve under very low ether tension. The right vagus was sectioned, the left vagus was intact. Under a high ether tension such a long period of inhibition would probably have proven fatal owing to failure of the circulation. This record shows that while under very light ether tension it is rarely possible to inhibit respiration for a long period of time, a fatal result does not occur.

For some unknown reason, ether does not abolish the reflexes which inhibit respiration so long as the respiratory center responds. Under deep etherization the threshold of the cells of the respiratory center is greatly increased to the chemical stimuli. A point is thus reached at which the irritability of the center is so depressed by ether that it will not respond to the increasing amounts of carbon dioxide in the blood or to the excitatory reflexes, but will respond to the inhibitory reflexes. At this time stimulation of inhibitory fibers will produce death and, owing to the action of the high tension of ether on the circulation, a very short period of asphyxia will produce death very quickly. The ether tension under which death will follow the stimulation of the reflexes inhibiting respiration is fairly constant, although individual variations occur.

There seem to be factors other than deep etherization, although probably minor ones, involved in these experiments. The reflex producing inhibition of respiration seems very resistant to agencies which usually depress or abolish the excitatory reflexes of respiration. Thus it seems quite possible that most conditions which decrease the irritability of the respiratory center might allow death to occur by reflex inhibition of respiration providing the circulation were also depressed. The production of deep anesthesia, although probably the most common and potent, would be only one of these agencies.

The accumulation of the data substantiating the idea that depression of the respiratory center associated with a depressed circulation is the important factor is not complete and further investigation in regard to it is being carried on. The following suggestive facts, however, have been obtained: The inhibitory reflex of respiration is decreased or completely abolished during periods of hyperpnea; the period of apnea following hyperpnea under light ether anesthesia is not increased by the stimulation of the nerves which inhibit respiration, and the inhibitory reflex is decreased or abolished during the period of increased respiratory movements in the first stage of asphyxia. An animal cannot be killed by reflex inhibition of respiration during the first stage of asphyxia. However, as asphyxia is prolonged and the respiratory movements begin to decrease, stimulation of inhibitory nerves will inhibit respiration and in many experiments produce sudden death. In some experiments, while the time during which respiration is inhibited under deep ether is greatly increased over that under light ether, death cannot be produced. A slight period of asphyxia will increase the time of inhibition so that death will occur. It would seem that the lack of oxygen may be a factor. Inhibition of respiration under deep etherization frequently produces death very quickly. The blood pressure usually decreases at once and the heart soon stops beating. This result would seem to imply that death was due to or associated with other factors than asphyxia. However, a comparison

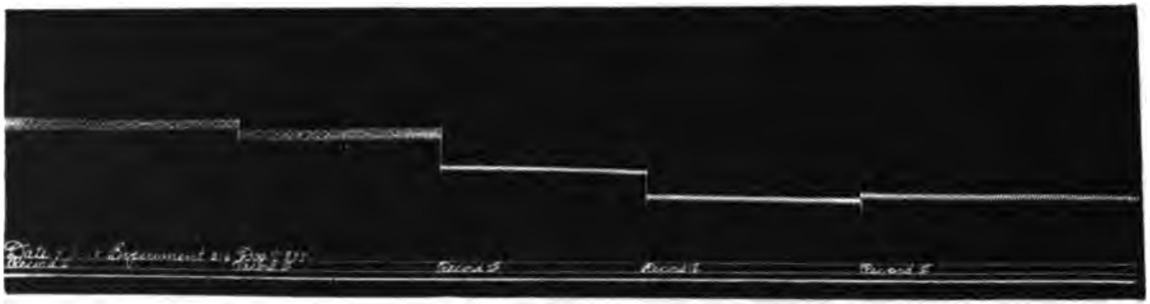


Fig. 9. Photograph of kymograph record showing a typical result following ligation of all the structures in the limbs except the major artery. Animal etherized at 8:46. Method—anesthetometer. Apparatus arranged to record carotid blood pressure, major artery to each limb exposed. Record 1 taken at 9:25, blood pressure 160. Ligatures were applied to each limb including all structures except the major artery. Record 2 taken at 9: 40, blood pressure 150. Record 3 taken at 10:40, blood pressure 114. Record 4 taken at 11:40, blood pressure 80. Animal developing the signs of shock. Ligatures removed at 11:44. Record 5 taken at 12:15, blood pressure 88.

of the curves in instances in which death was due to asphyxia under deep ether shows a close similarity to those found in the condition produced experimentally. The asphyxia under deep ether seems to explain the sudden decrease in blood pressure and stoppage of the heart, although an active process may be involved.

Under light surgical anesthesia respiration is seldom inhibited for a very long time. In a few of our experiments stimulation of the superior laryngeal nerves under an ether tension, slightly too low for surgical work, has produced a partial inhibition of respiration for as long as four to eight minutes. This period of inhibition is longer than that which produced death in several of the experiments under deep ether. Under the very low ether tension, however, blood pressure remained practically normal and death did not occur. Undoubtedly, if a high ether tension had been used, death would have followed such prolonged periods of inhibition. These results would furnish some support to the idea that the sudden death following inhibition of respiration under a high ether tension was mainly owing to a failure of the organism to compensate for the asphyxiation when saturated with a high ether tension. This relation of asphyxia to deep etherization has been previously discussed by Gatch, Gann and Mann (4). The important fact

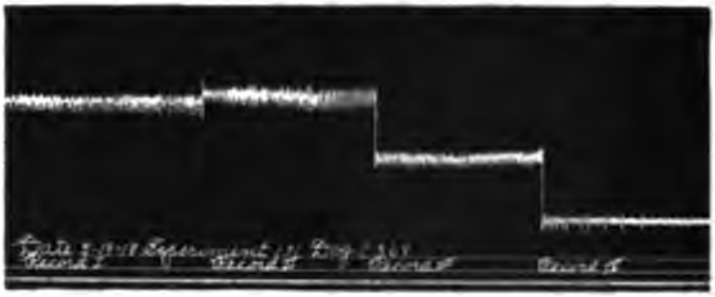


Fig. 10. Photograph of kymograph record showing a rapidly fatal termination following venous obstruction to all the limbs. Animal etherized at 8:30; method --modified McGrath. Apparatus arranged to record carotid blood pressure. Major artery to each limb exposed. Record 1 taken at 8:55, blood pressure 130. Ligatures were immediately applied to each limb including all structures except the major artery. Record 2 taken at 9:06, blood pressure 140. Record 3 taken at 10:00, blood pressure 86. Animal beginning to exhibit signs of shock. Record 4 taken at 10:30, blood pressure 34. The ligatures were now removed but the animal died during this procedure.

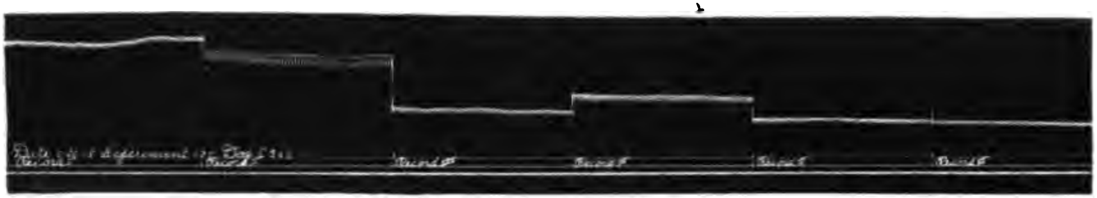


Fig. 11. Photograh of kymograph record showing slight recovery after removal of ligatures. Animal etherized at 1:19; method—modified McGrath. Apparatus arranged to record carotid blood pressure. Major artery to each limb exposed. Record 1 taken at 1:55, blood pressure 135. Ligatures were applied to each limb including all structures except the major artery immediately after taking this record. Record 2 taken at 2:05, blood pressure 120. Record 3 taken at 3:00, blood pressure 65. Animal exhibited signs of shock. The wire ligatures were removed at 3:02. Record 4 taken at 3:15, blood pressure 78. Record 5 taken at 4:00, blood pressure 50. Record 6 taken at 4:45, blood pressure 50.

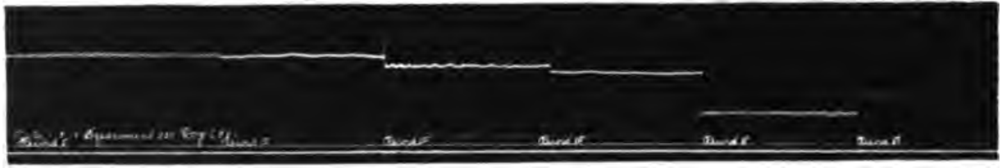


Fig. 12. Photograph of record showing a sudden delayed drop in blood pressure and partial recovery after removal of the ligatures. Animal etherized at 8:30; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 9:05, blood pressure 120. Ligatures were applied to each limb including all structures except the major artery between 9:10 to 9:15. Record 2 taken at 9:15, blood pressure 120. Record 3 taken at 10:15, blood pressure 102. Record 4 taken at 11:15, blood pressure 92. Animal beginning to exhibit signs of shock. Record 5 taken at 12:15, blood pressure 36. Ligatures were removed immediately after taking record 5. Record 6 taken at 12:40, blood pressure 60.

shown in these experiments is that the inhibitory reflex of respiration under ether anesthesia persists as long as respiratory movements occur.

Experiments dealing with the attempt to produce death by stimulating nerves other than electrically, and under conditions other than deep etherization, are not complete. However, it would seem quite possible to produce a condition of depression of the respiratory center by asphyxia, oxygen-lack or other methods, without otherwise greatly disturbing the general condition of the animal, so that the stretching of a nerve or the pulling of the mesentery might produce a serious or fatal condition owing to inhibition of respiration.[2]

What practical bearing such experiments have on the shock problem, it is impossible to state at the present time. Death is produced so suddenly and strikingly under these conditions that it seems highly probable the same effects have occurred in man.[3] It is quite possible that deep etherization and inhibition of respiration, while operations are being performed in the region of the neck, axilla and diaphragm, regions in which traumatic procedures are prone to produce stoppage of respiration, are responsible for some of the sudden deaths on the operating table which the surgeon has diagnosed as due to shock. It should be noted that the depth of anesthesia at which inhibitory reflexes become dangerous is no greater than that which some surgeons employ.

SUMMARY

Ether tensions that will decrease or abolish the excitatory reflexes of respiration do not seem to depress the inhibitory reflexes, and in most instances the action of the inhibitory reflex seems to be increased, although this may be only a relative result. Ether tensions that will depress the respiratory center so that it will not respond to the increase of carbon dioxide in the blood, usually will not abolish the inhibitory reflex. Under such conditions stimulation of the nerves inhibiting respiration will quite frequently produce death. This may be the process by means of which sudden death is produced during operation. However, death due to inhibition of respiration should never occur under light surgical anesthesia.

---

[2] In an excellent article on shock, Webster (5) shows a kymograph tracing in which pulling on the mesentery in a shocked animal produced inhibition of respiration and death.

[3] Hewitt (6) reports a case in which respiratory failure occurred during abdominal incision, but artificial respiration was effective. The corneal reflex had disappeared. Results in this case seem to parallel our experiment.

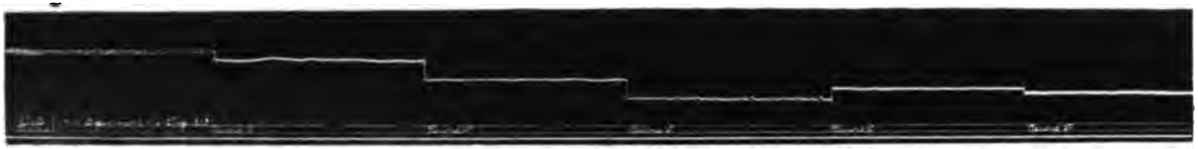


Fig. 13. Photograph of kymograph recorded showing slight recovery and then failure after removal of the ligatures. Animal etherized at 9:25; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 9:45, blood pressure 128. Ligatures were applied to each limb including all structures except the major artery between 9:51 to 10:00. Record 2 taken at 10:00, blood pressure 108. Record 3 taken at 11:00, blood pressure 76. Animal beginning to exhibit the signs of shock. Record 4 taken at 11:30, blood pressure 45. The ligatures were removed at 11:35 to 11:40. Record 5 taken at 12:10, blood pressure 60. Record 6 taken at 1:10, blood pressure 50.

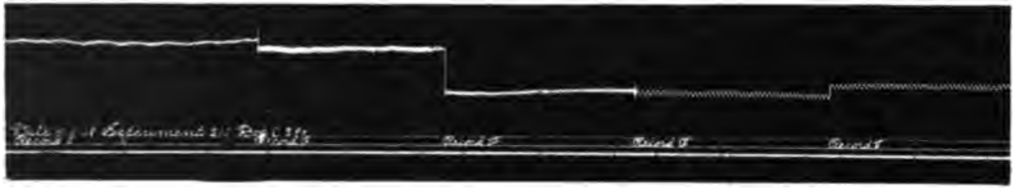


Fig. 14. Photograph of kymograph record showing partial recovery after removal of the ligatures. Animal etherized at 2:25; method—anesthetometer. The apparatus was arranged to record carotid blood pressure. The major artery to each limb was exposed. Record 1 taken at 3:04, blood pressure 118. Ligatures were immediately applied to each limb including all structures except the major artery. Record 2 taken at 3:15, blood pressure 110. Record 3 taken at 3:45, blood pressure 60. Animal beginning to exhibit the signs of shock. Record 4 taken at 4:45, blood pressure 56. Ligatures were removed immediately after taking this record. Record 5 taken at 12:15, blood pressure 88.

IV. THE RELATION OF THE CAPILLARY AND VENOUS BEDS TO THE SIGNS OF SHOCK

This investigation was made for the purpose of determining the smallest capillary and venous area which could be made to contain enough fluid to produce the signs of shock. There is no doubt that the cause of the condition which the surgeon calls shock is, in a large number of cases, a loss of circulating fluid. The method by means of which this fluid is lost to the circulation is not known nor is its place of sequestration fully established. It has been shown (7) that the capacity of the vascular system in the splanchnic area is such that it would hold several times the normal amount of blood. There is also no doubt but that in shock due to exposure of the abdominal viscera the initial loss of fluid takes place in this area. What relation the remaining capillary and venous area of the body bears to the loss of circulating fluid is not known. Cannon (8) believes, and has presented data to substantiate the belief, that the lost fluid is due to capillary stagnation.

Janeway and Jackson (9) have shown that a circulatory failure which presents the typical signs of shock can be produced in dogs by a partial occlusion of the inferior vena cava at its point of entrance into the thorax. This has been corroborated by other observers.

The method of investigation in this series of experiments consisted in including in one single strong ligature all the structures to each limb except the major artery. In this way the major artery was allowed to pump blood into the limb from which all venous and lymph return was obstructed. The animal was maintained under a constant ether anesthesia and carotid blood pressure was recorded (mercury manometer). The ligatures were always applied so as to include as much of the limb as possible. Under such experimental conditions three results might occur: *a*, Stagnation of circulatory fluid in the occluded venous and capillary area to their full capacity. The general effects of this loss of fluid would depend on the size of the area involved and the ability of the remainder of the tissues to compensate for the loss. *b*, Injury, owing to lack of proper circulation, to all the tissues of this region. *c*, After removal of the ligatures, the passage into the general circulation of toxic products which might have been formed during the period of occlusion.

The results of a large series of experiments are in general agreement. The first result of ligating the limbs in the manner described is usually a slight and transient rise in blood pressure, although occasionally the pressure may decrease from the beginning. In any event, the blood pressure soon decreases and at the end of two hours has only about one-half its initial value. Usually this decrease takes place very gradually but sometimes it drops suddenly after having been maintained at approximately its normal level for a long time. Other signs of shock also develop; for example, a short time after the application of the ligatures to the limbs it is usually possible to decrease the ether tension to a point greatly below that necessary to maintain anesthesia in a normal animal. Thus, at the end of two hours after ligation, an animal usually exhibits the major signs of shock.

If the ligatures are removed after blood pressure has decreased about one-half of its initial value, one of two results follows. Blood pressure either increases or decreases. In the majority of our experiments blood pressure increased after the removal of the ligatures, but complete recovery did not occur. This rise in pressure was seldom long maintained but soon decreased again and within an hour or so was as low as when the ligatures were removed. In some of the experiments the blood pressure fell immediately after the removal of the ligatures. In most of the animals death soon occurred although in a few the blood pressure was maintained at that low level for a long period of time. The results of the experiments may be best explained as follows: The initial fall in blood pressure is probably due to the occlusion and stagnation of circulatory fluid in the isolated capillary and venous areas. The variation in the length of time in which the blood pressure decreases apparently depends on the ability of the rest of the body to compensate for the fluid loss. The variable results following the removal of the ligature are due to damage to the tissues in the involved area and the passage of toxic products from the injured tissues into the general circulation.

The primary factors involved in these experiments, *a*, stagnation of circulatory fluid, *b*, damage to large areas of tissue in such a manner that their mechanism for controlling fluid exchange and, when the area is large enough, volume is impaired, and *c*, toxic products of cell metabolism and cell disintegration may all be of importance in the production of some of the conditions which the surgeon calls shock.

In such experiments it is difficult to make estimations of the relative capacity of the capillary and venous beds involved to the total capacity of all these beds in the body. However, it was possible to obtain approximate data on the relation of the total weight of tissue below the ligature to the total body weight. This was done in animals not subjected to ligation in order to eliminate the possibility of changes in the tissues themselves as the means of complicating the results. The animals had been used in other experiments and had been fasted for twelve hours before death. The limbs were amputated at the level at which the ligature was applied and a comparison of the total weight of the amputated limbs to total body weight was made. As there are several sources of error, the comparisons are only approximate. The data show that on an average 15 per cent of the total weight of the tissues of the body was involved in the ligature.

Ligation of only three limbs produced variable results. Blood pressure was rarely lowered to a shock pressure. Section of the nerves to each limb did not seem to produce any change in the effect of the ligation.

In summarizing it may be restated that ligation of all the structures to the limbs of a dog except the major artery, will usually produce all the signs of shock. The relative amount of tissues involved by these ligatures was on an average approximately 15 per cent of the total body weight. The experiments show that a circulatory impairment following venous obstruction of the return of blood from the four limbs of an etherized animal is sufficient to produce the signs of shock.

BIBLIOGRAPHY

(1) **Mann**: Amer. Journ. Surg., Anes. Suppl., 1917, xxxi, 107.
(2) **Connell.**: Surg., Gynec. and Obst., 1913, xvii, 245.
(3) **McGrath**: Journ. Amer. Med. Assoc., 1913, lxi, 1516.
(4) **Gatch, Gann and Mann**: Journ. Amer. Med. Assoc., 1913, lx, 1273.
(5) **Webster**: Canadian Med. Assoc. Journ., 1913, iii, 98.
(6) **Hewitt**: Anesthetics and their administration, London, 1912, 590.
(7) **Erlanger, Gesell, Gasser and Elliott**: Journ. Amer. Med. Assoc., 1917, lxix, 2089.
(8) **Cannon**: Journ. Amer. Med. Assoc., 1918, lxx, 611.
(9) **Janeway and Jackson**: Proc. Soc. Exper. Biol. and Med., 1914, xii, 193.

THE PHOTO-CHEMICAL EFFECT OF CERTAIN FLUORESCENT SUBSTANCES ON RENNIN

JANET HOWELL CLARK

From the Pathological Laboratory of the Johns Hopkins University

Received for publication September 13, 1918

INTRODUCTION

Von Tappeiner, Jodlbauer and their coworkers (1) have carried out a great number of experiments on the destructive effect of visible radiation on various cells, enzymes and toxins when fluorescent substances are present. Not all fluorescent substances are found to be consistently harmful and there is no correlation between the degree of fluorescence and the amount of photodynamic action for the different materials used. However, the fact that all the substances which were found harmful are fluorescent in some degree seems to establish a definite connection between the two effects. Two facts that stand out clearly are *a*, that only the wave-lengths absorbed by the fluorescent substance are harmful; *b*, that for visible rays the presence of oxygen is necessary to produce photodynamic action. The first fact would be true if the effect were due to any photochemical change and does not necessarily point to any connection with fluorescence. The second fact seems to indicate that the effect is photochemical and that fluorescence is not the essential factor, since oxygen is not necessary for fluorescence.

A number of experimenters have objected to the conclusion that the property of fluorescence is in itself harmful. Straub (2) found that the oxidation of many well-defined chemical substances, by the oxygen of the air, is greatly increased by eosin in the presence of light. He concluded that eosin is toxic on account of poisonous substances formed by autoxidation. Heffter (3) examined the acids formed by eosin in the process of autoxidation and found carbonic, oxalic, phthalic and hydrobromic acids. It would seem that the formation of these acids might easily explain the harmful effects on paramoecia and bacteria, especially in view of the Ledoux-Lebard experiment and von Tappeiner's addition to it. Ledoux-Lebard (4) found that a previously illuminated solution of eosin was fatal to paramoecia in the dark, whereas a freshly prepared solution of the same strength was harmless. Von Tappeiner showed that this effect is definitely due to the formation of acids, as neutralizing the illuminated solution renders it harmless again. He is, however, convinced that the slow formation of free acids does not account for the swift photodynamic effect observed, especially as paramoecia will die in neutral and alkaline solutions in the presence of fluorescent substances and light. Also, a photodynamic death is followed by a swift disintegration of the paramoecia, whereas after a death from free acids they keep their shape for many hours.

Von Tappeiner's own theory attempts to explain the fact that while oxygen is necessary for a toxic effect with visible light, ultra-violet rays are harmful in its absence. He believes that the fundamental phenomenon, in both cases, is a decomposition by which groups are released which can be oxidized by light in the presence of oxygen. With short wave-lengths this decomposition is far-reaching under all conditions whereas, with visible light, it only reaches a perceptible degree when oxygen is present and the oxidizable decomposition products are continually removed by oxidation. The fluorescent substances, he believes, hasten the oxidation and therefore the whole process.

This explanation still leaves the rôle of the fluorescent substances in a very vague state and the following experiments were carried out in hopes of finding some clue as to what this rôle might be. To avoid complications from the toxic effects of acids, rennin was chosen as the subject for experimentation.

Rennin is favored by a slightly acid medium and is not killed by heat under a temperature of 55°C.

PREVIOUS WORK ON RENNIN

Without the presence of a sensitizing substance rennin is very sensitive to ultra-violet light and is affected very little if at all by visible rays. Emmerling (5) found that an exposure of five days to indirect sunlight cuts down the activity of rennin to one-half, exposure to direct sun to one-third. Hertel (6) found that the waves of λ (wave length) $280\mu\mu$ are very destructive to rennin and Schmidt-Neilson (7) found that 96 per cent of the total effect of a quartz mercury arc on rennin is due to waves of λ $(220 \rightarrow 250)\mu\mu$.

Riegner (8) and Quiring (9), two pupils of V. Tappeiner, investigated the effect of light on rennin in the presence of fluorescent substances. They found a strong inhibitive action in the presence of substances of the fluorescein group and a measurable, though small, effect with the anthracene group, magdala red and methylene blue. Negative results were obtained with substances of the acridine, phenazin and napthalene groups, with quinoline dyes and aesculin.

Huber (10) in a paper on the sensitizing effect of fluorescent substances, gives some interesting experiments on the effect of eosin and erythrosin on rennin ferment. He gives the following results:

1. While a given rennin solution, containing eosin or erythrosin, is unaffected as long as it remains in the dark and will clot milk in ten minutes, on exposure to light its activity is decreased so that the clotting does not occur for an hour or more.
2. The same result, though to a very much smaller degree, was observed when the eosin or erythrosin was added to milk and exposed and the rennin added subsequently.
3. When the eosin or erythrosin was exposed alone, and then added to the milk and rennin, no weakening of activity was observed.
4. The presence of air favored the toxic effect of eosin and light but there was still a large effect when air was excluded.
5. Under screens of eosin and erythrosin solutions the effect of light is diminished but still large.
6. The toxic effect of light on eosin and rennin is permanent, since a solution exposed and kept overnight has the same action as one to which milk is added immediately after exposure.

METHOD

In the following experiments a solution was made from one Hansen's junket tablet to 30 cc. of distilled water. To 5 cc. of this solution definite amounts of solutions of eosin and other substances were added. The solution was exposed to direct sunlight in an open beaker and then added to 15 cc. of milk at a temperature of 30°C. The mixture was placed in a thermostat at 37°C. and the time of clotting observed. The controls of 5 cc. exposed junket solution and 15 cc. of milk clotted in 5 → 8 minutes.

In experiments with color screens of absorbing solutions, the rennin solution was placed in a small flat dish and a slightly larger dish, covered and filled with the absorbing solution, was placed on top. The sides of the bottom dish were blackened so that the light came only through the absorbing screen. With colored glass screens the upper dish was replaced by the glass.

RESULTS

The effect of heat was first tried and the results are given in table 1. There is therefore no destructive effect from heat under 55°C. and as the experiments were conducted in cool weather the temperature, even in direct sunlight, was always under 30°C. and there were no complications from heat.

TABLE 1

Effect of heat

	MINUTES TO CLOT
5 cc. rennin solution heated to 50°C.	8
5 cc. rennin solution + 0.0005 gram eosin heated to 50°C.	5
5 cc. rennin solution heated to 55°C.	8
5 cc. rennin solution + 0.0005 gram eosin heated to 55°C.	5
5 cc. rennin solution heated to 60°C.	No clot in 35 minutes
5 cc. rennin solution + 0.0005 gram eosin heated to 60°C.	

The effect of acid, alkali and alcohol is shown in table 2.

TABLE 2

Effect of acid, alkali and alcohol

	MINUTES TO CLOT
5 cc. rennin solution	5→7
5 cc. rennin solution + 0.5 cc. 0.1 per cent NaOH	12
5 cc. rennin solution + 1.0 cc. 0.1 per cent NaOH	20
5 cc. rennin solution + 0.5 cc. 0.1 per cent H_2SO_4	4
5 cc. rennin solution + 1.0 cc. 0.1 per cent H_2SO_4	3
5 cc. rennin solution + 0.2 cc. 1 per cent H_2SO_4	2
5 cc. rennin solution + 0.4 cc. 1 per cent H_2SO_4	1
5 cc. rennin solution + 0.5 cc. 95 per cent alcohol	5
5 cc. rennin solution + 1.0 cc. 95 per cent alcohol	5

Since the activity of rennin is favored by an acid and hindered by an alkaline medium, and unaffected by alcohol, it was possible to try the effect of several substances that are soluble in alcohol and not in water.

The fact that eosin is harmless except on exposure to light is shown in table 3.

TABLE 3

Effect of eosin in dark

	MINUTES TO CLOT
5 cc. rennin solution in dark 5 hours	7
5 cc. rennin solution + 0.0005 gram eosin in dark 5 hours	7
5 cc. rennin solution + 0.001 gram eosin in dark 5 hours	7
5 cc. rennin solution + 0.0005 gram eosin in dark 48 hours	8
5 cc. rennin solution + 0.001 gram eosin in dark 48 hours	8

Huber (10) found that previously illuminated eosin is harmless and that the toxic effect resulting from exposure of eosin and rennin is permanent. These conclusions are confirmed by the results given in tables 4 and 5.

TABLE 4

Effect of previously illuminated eosin

	MINUTES TO CLOT
0.001 gram eosin (water solution) 3½ hours sun (rennin added later).	6
0.001 gram eosin (water solution) 1¾ hours sun (rennin added later).	7

TABLE 5

Permanency of toxic effects

MILK ADDED AT ONCE	TIME TO CLOT	MILK ADDED AFTER STANDING OVER NIGHT	TIME TO CLOT
5 cc. rennin solution + 0.0005 gram eosin, 1 hour sun	1¾ hours	Same	1¾ hours
5 cc. rennin solution + 0.001 gram eosin, 1 hour sun	3½ hours	Same	3¼ hours
5 cc. rennin solution + 0.001 gram fluorescein, 2½ hours sun	50 minutes	Same	50 minutes
5 cc. rennin solution + 0.001 gram methylene blue, 2½ hours sun	1½ hours	Same	2 hours

After these preliminary experiments special ones were undertaken with a view to determining what rôle fluorescence plays in the effect.

If fluorescence is the determining factor one would expect the following results:

1. All fluorescent substances should be harmful, when wave-lengths capable of stimulating fluorescence are used, and non-fluorescent substances should be harmless.

2. A strongly fluorescent substance should be more toxic than one in which the fluorescence is weak or barely perceptible.

3. The wave-lengths of light which stimulate maximum fluorescence should give the maximum toxicity.

To test the first point a number of substances were examined. The following gave negative results: Congo red, methyl green, fuchsine, neutral red, methyl orange, chlorophyll (alcoholic solution), quinine sulphate (faintly acid solution), gentian violet, resorcinol and haematoporphyrin. Of these, chlorophyll and quinine sulphate show strong fluorescence; congo red, neutral red and haematoporphyrin weak fluorescence. Toxicity in the presence of light was found to be very marked for eosin and erythrosin and present, though weak, for fluorescein and methylene blue all of which are fluorescent. It was also found, as will be given in detail later, that a non-fluorescent emulsion of silver bromide and rennin solution was toxic in the light. So all fluorescent substances are not harmful and all non-fluorescent substances are not harmless.

As to the second point, experiments have been carried out by V. Tappeiner (1) on the relation between the degree of fluorescence and the amount of toxicity for paramoecia. He found that, with any given substance, the toxicity changed in the same direction as the fluorescence. For example, they both decrease with the addition of salt to quinine sulphate. But, except for this, there was no connection. Fluorescein is very fluorescent and not very harmful while the opposite is true of anthraquinone.

And, in the case of substances of the fluorescein group, the toxicity actually increases with decrease in fluorescence as is shown in the following table from Von Tappeiner's work (11) (table 6).

I obtained a similar result with the fluorescein groups and rennin (table 7).

Fluorescein shows even less toxicity than methylene blue, a weakly fluorescent substance of another group (see table 8).

So far there would seem to be no evidence that fluorescence is the controlling factor in photodynamic action on rennin. The third point to be tested is, however, the decisive one and, in the case of eosin, the wave-lengths giving maximum fluorescence are well known. Nichols and Merritt (12) made a careful study of the fluorescent spectra of a number of substances with the spectrophotometer in order to test the validity of Stokes' law (i.e., the fluorescent light is always of greater wave-length than the exciting light). Among these substances they tried eosin in dilute alcoholic solution. Their curves showing the distribution of intensities in the fluorescent spectrum, for different wave-lengths of exciting light, are reproduced in figure 1. The maximum wave-length of the fluorescent light and the distribution of intensity in the fluorescent band, are independent of the wave-length of the exciting light, but the maximum intensity of fluorescent light for eosin is given

TABLE 6

SUBSTANCE	BRIGHTNESS OF FLUORESCENCE	TOXICITY TO PARAMOECIA IN DIFFUSE DAYLIGHT (THAT IN DARK = 1)	TOXICITY TO INVERTIN IN PER CENT
Fluorescein	Very strong	11	0
Tetrachlor fluorescein	Strong	35	10
Tetrabrom fluorescein (eosin)	Moderate	60	57
Tetraiodo fluorescein (erythrosin)	Weak	80	89
Dichlortetrabrom fluorescein	Only perceptible with lens in sun	150	89
Dichlortetraiodo fluorescein		100	96
Tetrachlortetraiodo fluorescein		170	89

TABLE 7

	EXPOSURE TIME	
	15 minutes	45 minutes
	TIME TO CLOT	
5 cc. rennin solution + fluorescein 0.001 gram	8 minutes	12 minutes
5 cc. rennin solution + erythrosin (tetraiodo fluorescein) 0.001 gram	12 minutes	45 minutes
5 cc. rennin solution + eosin (tetrabrom fluorescein 0.001 gram	15 minutes	No clot in 6 hours

TABLE 8

	EXPOSURE TIME	
	1½ hours	2½ hours
	TIME TO CLOT	
5 cc. rennin solution + fluorescein (0.001 gram)	20 minutes	50 minutes
5 cc. rennin solution + methylene blue (0.001 gram)	30 minutes	1¾ hours

by λ 590 + μμ for the exciting light which is greater than 580μμ, the wave-length of maximum intensity of fluorescent light (a contradiction of Stokes' law). With decreasing wave-lengths the intensity of the fluorescent light diminishes.

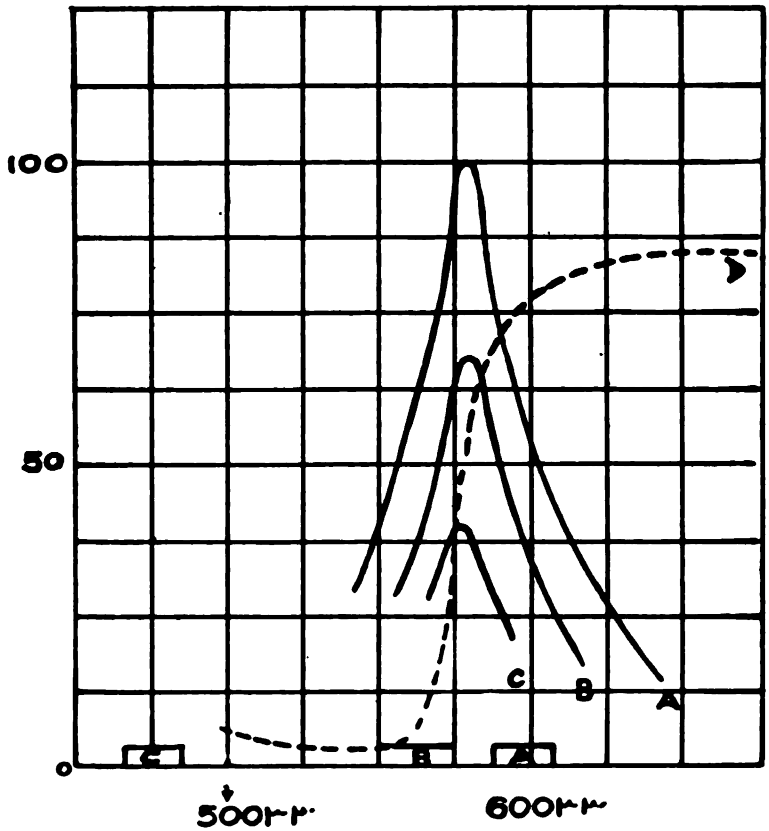


Fig. 1. The fluorescence spectrum of eosin when the exciting light lies in different regions of the spectrum. Ordinates give the intensity of fluorescence and abscissae the wave-lengths. Curve *A* was obtained when the exciting light was confined to the region marked *A* on the axis of wave-lengths. *D* = Transmission curve of eosin. Vertical scale arbitrary. Horizontal scale in μμ = 0.000001 mm.

Huber, in his experiments with rennin and eosin, found that an eosin screen did not cut down the effect of light to any great extent. This is interesting as, in general, for any photochemical or fluorescent effect, one would expect only the wave-lengths absorbed by the substance itself to be effective. However, since the results of Nichols and Merritt show that the maximum fluorescence of eosin is caused by wave-lengths just to the red of the absorption band, the results given by Huber may not be in disagreement with the theory that fluorescence is the controlling factor in photodynamic action on rennin. In order to find just which wave-lengths are most injurious, 5 cc. of rennin solution + 0.001 gm. of eosin or erythrosin, was exposed to sunlight behind screens of methyl red, potassium bichromate, chrome alum, cuprammonium and eosin (or erythrosin). These substances have the following transmission in the visible.

Methyl red	λλ 600 μμ → 690 μμ
Potassium bichromate	λλ 500 μμ → 690 μμ
Chrome alum	λλ 450 μμ → 550 μμ
Cuprammonium	λλ 420 μμ → 520 μμ

Eosin, in moderately dilute solutions, cuts off everything below λ 550μμ in the visible, but, with a very dilute solution, there is partial transmission from λ 460μμ to λ 420μμ.

Erythrosin, moderately dilute, cuts out everything below λ560μμ but, when more dilute, gives weak transmission from λ420μμ to λ480μμ.

The glass screens used were more dense than the solution screens and required longer exposure. They showed the following transmission:

Red glass	λλ 680 μμ → 600 μμ
Yellow glass	λλ 680 μμ → 510 μμ
Green glass	λλ 620 μμ → 480 μμ
Blue glass (cobalt)	λλ 510 μμ → 420 μμ
With a faint transmission band at	λ 600 μμ

If the effect is one due to fluorescence the maximum effect for eosin would be expected behind the potassium bichromate screen. There is unfortunately no definite investigation giving the maximum stimulating wave-length for erythrosin. It has a yellowish-green fluorescence and the stimulating wave-lengths would probably lie in the green and blue. Experiments with the color screens give the results of tables 9, 10, 11 and 12.

As eosin has no absorption band in the ultra-violet, one would expect no greater inhibition in that region with eosin and rennin than with rennin alone. Experiments with a quartz mercury arc confirmed this.

No color screen experiments were tried with fluorescein and methylene blue as they showed such a small effect. With erythrosin and eosin the maximum inhibition was found in the blue and green, green

TABLE 9

Eosin (0.001 gram in 5 cc. rennin solution) solution screens

	EXPOSURE TIME							MEAN
	2 hours sun	2 hours sun	1½ hours sun	1½ hours sun	1½ hours sun	1½ hours sun	1½ hours sun	
	Clotting time (minutes)							
Red screen	13	5	10	12	12	12	13	11
Yellow screen	19	9	30	22	60	20	30	27
Green screen	25	13	45	45	240	110	60	77
Blue screen	90	35	50	60	300	85	210	119
Eosin screen	35	28	15	15	12	15	18	20

TABLE 10

Eosin (glass screens)

	EXPOSURE TIME				MEAN
	2½ hours sun	3 hours sun	2 hours sun	2 hours sun	
	Clotting time (minutes)				
Red	10	9	9	9	9
Yellow	10	15	10	11	11
Green	90	50	20	20	45
Blue	270	180	25	25	125

TABLE 11

Erythrosin (0.001 gram in 5 cc. rennin solution) solution screens

	EXPOSURE TIME								MEAN
	1½ hours sun	1½ hours sun	3 hours moderate sun	3½ hours moderate sun	4 hours sun	1½ hours bright, 2 hours dull	2½ hours sun	2 hours sun	
	Clotting time (minutes)								
Red	15	10	8	13	30	20	8	12	15
Yellow	15	10	10	15	55	30	35	30	25
Green	20	10	18	35	170	30	190	105	72
Blue	18	15	15	25	210	38	70	60	56
Erythrosin	18	10	10	13	40	18	30	35	22

light being most effective for erythrosin and blue for eosin. In the case of erythrosin this would be consistent with the theory that fluorescence is the primary cause of inhibition. The results with eosin, however, show very definitely that this is not true as the most marked inhibition is due to blue light, which gives but faint fluorescence, and the yellow light that stimulates maximum fluorescence is almost harmless. No attempt was made to determine the relative amounts of energy transmitted by the different screens.

TABLE 12

Erythrosin (glass screens). Exposure time 4 hours sun

	CLOTTING TIME (MINUTES)					MEAN
Red	5	5	8	10	8	7
Yellow	10	8	8	20	8	11
Green	25	10	30	15	16	19
Blue	15	12	20	25	12	17

DISCUSSION

There is unfortunately no generally accepted theory of fluorescence. The most comprehensive one so far proposed is that first suggested by Wiedemann (13) in 1889, and later modified and extended by Wiedemann and Schmidt (14). According to this theory some chemical or physical change is produced in a luminescent substance during excitation and the emission of light is an accompaniment of a more or less gradual restoration of the modified substance to its original condition. That is, under the action of light, a portion of the substance is changed from a stable condition A to an unstable condition B. Luminescence may be due to the vibrations set up during the change A → B or to the fact that the reaction B → A proceeds, during excitation, with the emission of light. According to this theory fluorescence is primarily a photochemical phenomenon. It would seem reasonable to suppose that, while in the unstable state B, some fluorescent substances might be harmful and some not, depending on what by-products were formed in the changes $A \rightleftarrows B$. In this case one might look for a harmful photochemical effect in both fluorescent and non-fluorescent substances, but with the chances in the favor of effective substances being fluorescent, since all fluorescent materials are subject to photochemical action.

A very familiar photochemical reaction is that which takes place with a photographic plate. A plate coated with silver bromide alone.

and exposed to light is acted on to form free bromine but, when a certain amount is formed, a stationary balanced condition is reached owing to the recombination of silver and bromine. Such a plate would be of very little use in photography, being comparatively insensitive. If, however, a substance such as gelatine is present, which combines with the bromine as it is formed, a much greater decomposition of the silver bromide takes place.

Probably the action of light on eosin is entirely similar. When exposed to light it undergoes the transformation $A \rightleftarrows B$. Unless some substance is present to take up the harmful dissociation products as they are formed, the reaction soon reaches equilibrium and little, if any, permanently toxic substance is formed. This would explain the fact that previously illuminated eosin is harmless (see table 4). If however eosin and rennin are exposed together the rennin, acting like the gelatine of the photographic plate, takes up the toxic substances as they are formed and the reaction proceeds much further.

TABLE 13

	CLOTTING TIME
5 cc. rennin solution	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 3 hours dark	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 20 hours dark	10 minutes
5 cc. rennin solution + 0.5 gram AgBr 3 hours moderate sun.	1½ hours
5 cc. rennin solution + 0.5 gram AgBr 3 hours bright sun	No clot in 5 hours

Now eosin and erythrosin are both very toxic whereas fluorescein, from which they are derived, is comparatively harmless. Since eosin is tetrabrom fluorescein and erythrosin is tetraiodo fluorescein, the presence of the halogens must account for the difference. Probably the action of light results in freeing bromine and iodine, or some simple compound of these substances and these, being taken up by the rennin as they are formed, destroy its activity.

The experiment was tried of adding free bromine and iodine to the rennin solution and in both cases its activity was greatly inhibited, especially in the case of bromine where the smallest trace produced a strong effect. If the inhibition is due to the action of free halogens on rennin one would expect an emulsion of silver bromide to be harmful to rennin in the light and not in the dark. Experiments with silver bromide gave the following results which definitely confirm the view given above (table 13).

A direct proof of the formation of free iodine from erythrosin under the action of sunlight was tried by adding starch to a solution of erythrosin both with and without the rennin. The results however were negative as shown in table 14.

This probably means that in the case of erythrosin, the harmful dissociation product formed, under the action of sunlight, is not the free halogen but a toxic halogen compound.

TABLE 14

RENNIN SOLUTION	ERYTHROSIN	STARCH SOLUTION 2 PER CENT	RESULT (16 HOURS SUN)
cc.	*grams.*	*cc.*	
5	0.001	5	No evident darkening
5	0.010	5	No evident darkening
5	0.100	5	No evident darkening
5 (distilled water)	0.010	5	No evident darkening
5 (distilled water)	0.100	5	No evident darkening

CONCLUSION

These results seem to show that the inhibition of rennin by light is due to the formation of toxic substances as the result of photochemical action. This photochemical action may or may not be accompanied by fluorescence and fluorescence, always an evidence of photochemical action, may or may not be accompanied by the formation of toxic substances.

In the cases studied here the toxic effect was probably due to the formation of free halogens or toxic halogen compounds though doubtless other harmful substances are found in different photochemical reactions. Although the formation of free halogens or toxic halogen compounds accompanies the fluorescence of eosin and erythrosin, it is not directly associated with it, as shown by the color screen experiments with eosin. The change $A \rightleftarrows B$, which gives rise to fluorescence, is due primarily to the greenish-yellow and yellow light for which eosin shows the greatest absorption. The change which results in the formation of free halogens is apparently a subsequent $B \rightleftarrows C$ or accompanying $A \rightleftarrows C$ effect due primarily to greenish-blue and blue light for which eosin shows partial absorption. This accounts for the fact that, though strong solutions of eosin form an effective screen and keep the exposed solution from being inhibited, a weak solution, which lets through some radiation in the region λ 460$\mu\mu$ to λ 420$\mu\mu$, will allow considerable inhibition (see color screen results and Huber). In the absence of some substance, such as rennin, to take up C there is probably little reaction $B \rightleftarrows C$ or $A \rightleftarrows C$ present, and the chief photochemical change taking place is $A \rightleftarrows B$, which is accompanied by fluorescence.

BIBLIOGRAPHY

(1) **Von Tappeiner and Jodlbauer**: Die Sensibilisierende Wirkung Fluorescierender Substanzen: Gesammelte Untersuchungen über die Photodynamische Erscheinung, Leipsig, 1907. Also Ergebn. d. Physiol., 1909, viii, 698.
(2) **Straub**: Münch. Med.Wochenschr., 1904, Nr. 25.
(3) **Heffter**: Ber. d. Deutsch. Chem. Gessellsch., 1905. xxxviii, 3633.
(4) **Ledoux-Lebard**: Ann. d. l'Inst. Pasteur., 1902, xvi, 587.
(5) **Emmerling**: Ber. d. Deutsch. Chem. Gesell., 1901, xxxiv, 3811.
(6) **Hertel**: Biol. Zentralbl., 1907, xxvii, 510.
(7) **Schmidt-Nielsen**: Mitteil. aus Finsens Med. Lichtinst., 1904, ix.
(8) **Riegner**: Münch. Diss., 1904.
(9) **Quiring**: Münch. Diss., 1905.
(10) **Huber**: Arch. f. Hygiene, 1905, liv, 53.
(11) **Von Tappeiner**: Deutsch. Arch. f. klin. Med., 1904, lxxx, 427.
(12) **Nichols and Merritt**: Studies in luminescence, 1912; Phys. Rev., xviii, 403; Phys. Rev., xix, 18.
(13) **Wiedemann**: Wied. Ann., 1889, xxxvii, 177.
(14) **Wiedemann and Schmidt**: Wied. Ann., 1895, lvi, 177.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 312.

THE NATURE OF SENSORY STIMULATION BY SALTS

MARIAN IRWIN
Radcliffe College

Received for publication September 13, 1918

The purpose of this investigation is to determine as far as possible the nature of the effect of salt solutions on the chemical sense of the dung-worm, Allolobophora foetida. First of all it is necessary to show whether or not stimulation by some salts is dependent on the anions, since investigations so far have been in favor of cations only. It has been found that in the case of four salts the cations were responsible for stimulation; the order of their efficiency was as follows: $KCl < LiCl < NH_4Cl < NaCl$ (Parker and Metcalf, '06). In man, however, the sense of taste as regards salt solutions is found to be dependent upon cations in some instances and upon anions in others; for example, with NaCl, the chlorine ion is responsible for the salty taste, while with KCl, the action of the cation is masked by the potassium ion, which gives a bitterish taste. Therefore, on further investigation it may be possible to find that in dung-worms there may be anions that are efficient enough to mask the effect of the cations.

To carry out these tests an apparatus consisting of a small circular glass-table standing in a dish of the solution to be used in the test was employed (fig. 1). The top of the table, *T*, was 9 cm. in diameter, and was coated with paraffin. It was attached firmly to a stand, *S*, made of a small Petri dish 2.6 cm. in height, 3.5 cm. in diameter and filled with paraffin. This stand was fixed to the center of a large Petri dish, *D*, 15 cm. in diameter and 3.5 cm. in height. The paraffin used had a melting point of 62°C. and was tested for its acidity by the method devised by Haas ('16) for the determination of the output of carbon dioxide by plants. In preparing to test the worms, about 450 cc. of the liquid to be used was poured into the large Petri dish till its level, *L*, was within 3 mm. of the top of the table. A wet filter paper with a smooth surface was then placed on the table and the whole apparatus was put in a tin dish containing enough water to rise on the outside of the large Petri dish about 2.5 cm. The temperature of the room and of the whole apparatus was kept approximately constant and within one degree of 20°C. The worm was then gently placed on the table by means of a chopstick and allowed to crawl toward the edge and finally to enter the solution. The period intervening between the moment the prostomium of the worm entered the solution and that at which it was withdrawn was recorded by a stop-watch. This interval is called the reaction-time of the worm to the solution. After the worm had been once placed on the table, it was not touched by the experimenter till an observation had been made. After that it was removed from the table, rinsed in tapwater and kept between folds of wet filter paper in a tumbler until the next observation was to be made.

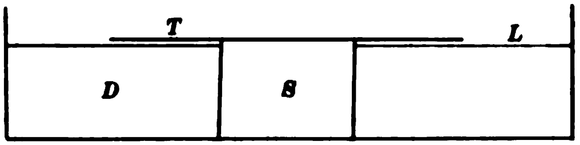


Fig. 1. Vertical section of the apparatus used in determining the worm's responses to various fluids. *D*, large Petri dish; *L*, level of fluids; *S*, stand; *T*, table covered with paraffin.

The various worms were found to differ in their reaction-times one from another to such an extent that it was impossible to draw directly any safe conclusions as to the relative efficiency of the different salt solutions without some method of standardization. A 0.2 molecular solution of KCl was therefore used as a standard solution, the reaction-time from it being regarded as 10 seconds. By this means variations due to the individual differences of the worms were largely eliminated.

To prevent the sequence in which the worms were tested and the order in which the solutions were used from distorting the results, the tests were made in the order of the numbers in the following scheme arranged for three worms and three solutions with an interval of fifteen minutes between observations to avoid as much as possible the after effect of the previous treatment with salt solution.

WORM DESIGNATED BY LETTER	STANDARD SOLUTION	TEST SOLUTION NO. 1	TEST SOLUTION NO. 2
A	1	2	3
	17	18	16
	24	22	23
B	6	4	5
	10	11	12
	27	26	25
C	8	9	7
	15	13	14
	19	20	21
	Averages	Averages	Averages

In obtaining a set of records by such a scheme as this, twelve worms of equal size were chosen, three to be used in each of four sets, whose averages were calculated separately. If the averages for the four sets agreed within the experimental error, they were averaged together and from such final averages curves were drawn.

The general method here outlined has the following advantages over methods used by previous experimenters. First, since the worm always enters the solution by extending the anterior end of its body, its prostomium, which is its most sensitive part, is always fully exposed to the solution. The records obtained are, therefore, records of the effect of the salts on the extended prostomium. Secondly, this method avoids handling the worm immediately before a reaction-time is taken. Thirdly, the response observed represents very closely a true reaction-time, since there are no mechanical obstructions for the worm to overcome as with the fence-method devised by Shohl ('14).

To test the efficiency of the anions, six potassium salts were chosen; namely, the nitrate, chloride, acetate, sulphate, tartrate and citrate. The acetate, tartrate and citrate were tested for their alkalinity by the following method. The standard solutions with a known amount of indicator, phenolsulphonephthalein, were made up by different mixtures of buffer solutions to represent different pH values. The same amount of the indicator was added to the test solution and the color was matched with the standard solutions. In this way it was possible to determine how much acid to add in order to bring the pH value to 7.0. The amount of acetic, tartaric or citric acid added for the neu-

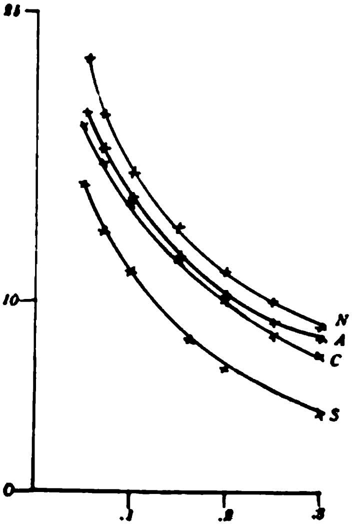


Fig. 2

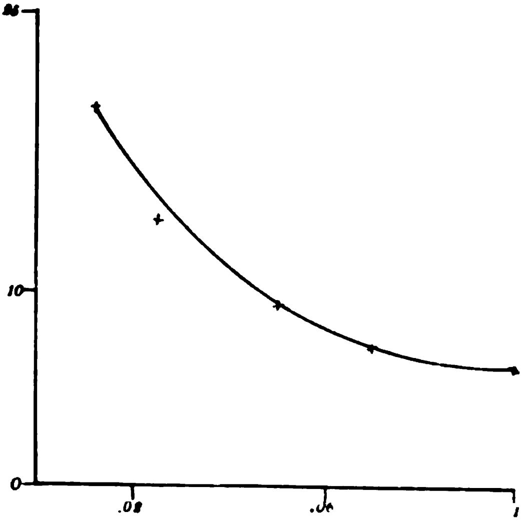


Fig. 3

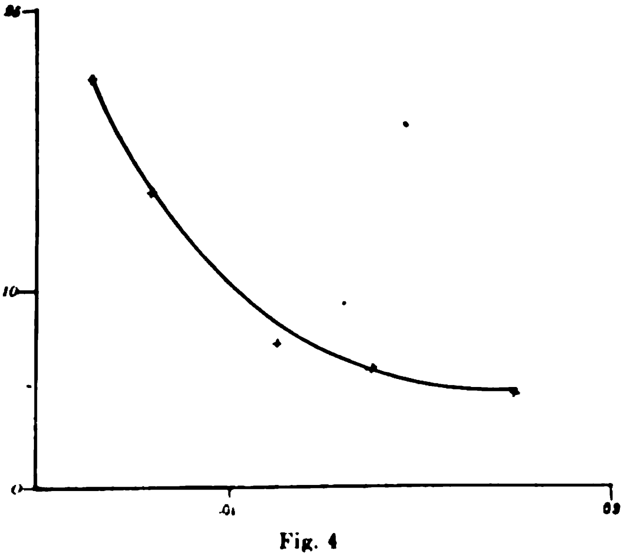


Fig. 4

Fig. 2. Relative efficiency of four potassium salts as stimuli for worms. The relative reaction-time in seconds of the worms to the solutions is applied as the ordinates and the molar concentrations as the abscissae. *N*, nitrate; *C*, chloride; *A*, acetate; *S*, sulphate.

Fig. 3. Effect of potassium tartrate. Relative reaction-time in seconds is applied as the ordinates and the molar concentrations as abscissae.

Fig. 4. Effect of potassium citrate. The relative reaction-time in seconds is plotted as the ordinates and the molar concentrations as abscissae.

tralization was not enough to change the dissociation percentage of the salts sufficiently to interfere with the experiments. Figures 2, 3 and 4 show the efficiency of the six potassium salts tested. The relative reaction-times in seconds are applied as ordinates and the molar concentrations as abscissae. At the concentration that brings about a relative reaction-time of about twenty seconds, the worms are fairly indifferent to the solutions, so that the relative reaction-times become too inconstant for an accurate computation. On the other hand at the concentration that gives the relative reaction-time of nine seconds, the reactions become so rapid that it is difficult to record them without considerable error. For this reason, in drawing comparisons of the efficiency of six salts it was necessary to choose concentrations which brought about relative reaction-times ranging from nine to sixteen seconds. On interpolating eight points between nine and sixteen seconds, and calculating the relative number of potassium ions from the dissociation percentages of a given salt, it is made evident that in case of nitrate, acetate, chloride and sulphate the number of potassium ions is approximately equal; therefore in these salts the potassium must be responsible for the stimulation. However, with the tartrate and the citrate, the amount of potassium ions is in all probability too small to attribute the effect to the cations.

The nature of the action of the solutions of potassium salts as dependent on the amount of the reacting substance present is susceptible of further proof as seen in the successive exposures of the worms to the potassium salt solutions, sucrose solutions and water (both tap and distilled). The only modification of the method described in the earlier part of the paper consisted in using for these tests a broader and deeper dish to hold the solution and a higher stand to carry the table. These changes prevented the worms from coming in contact with any part of the dish even when distilled water or a solution of sucrose was used, to which the worms react more or less indifferently. In making the tests, the worms were allowed to dip in and out of the solution in succession with an interval of less than ten seconds; the reaction-times, as in previous tests, were taken with a stop-watch. On comparing the reactions thus obtained with those to salt solutions, sucrose solutions and water, two types may be distinguished. One type is characteristic of those concentrations of the potassium salts that bring about an initial acceleration in the reaction-time, as indicated for 0.1 molecular potassium chloride solution in table 1. Here it will be seen that the reaction-times are at first long and become successively shorter and shorter.

TABLE 1

Reaction-time in seconds to successive applications of solutions of potassium chloride, of solutions of sucrose, and of distilled water. All fluids used were at 20°C. except the distilled water in the last series in the table; this was used at 33°C. The sign ∞ indicates that the worm entered the fluid instead of withdrawing from it.

	REACTION NUMBER																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0.1 M. KCl	45.0	6.2	4.8	1.8	3.0	6.8	1.8	2.4	4.0	0.6	0.4	1.0	0.8	1.8	0.4	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
0.01 M. KCl	∞	40.0	2.8	2.2	6.4	3.0	4.2	∞	3.8	3.2	2.8	1.8	3.8	6.8	8.0	5.0	8.0	7.0	13.0	∞	4.0	2.8	2.8	4.0
1 M. sucrose	11.0	8.8	3.8	3.0	4.0	6.2	2.0	4.0	2.2	1.8	3.2	2.6	2.8	3.0	3.0	1.8	3.4	1.8	1.0	1.8	2.4	2.2	1.0	2.0
0.5 M. sucrose	20.0	4.0	6.4	20.4	3.4	5.4	4.4	3.0	3.0	7.0	5.6	5.4	3.2	13.0	6.0	1.8	3.8	3.0	5.8	3.0	2.8	4.8	2.8	1.8
H_2O, 20°C.	7.0	4.6	4.0	2.0	1.8	1.2	3.0	1.8	1.4	2.6	8.8	13.0	2.0	1.8	1.8	2.0	2.0	1.8	4.0	4.2	1.8	1.0	2.2	3.0
H_2O, 33°C.	6.8	4.4	4.2	2.8	3.2	3.2	3.2	5.4	3.0	2.0	2.8	2.0	2.8	1.8	1.4	1.8	2.0	1.8	2.0	1.8	3.0	2.8	2.6	2.8

	REACTION NUMBER																						
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
0.1 M. KCl	0.2	0.2	0.2	0.2	0.2	0.2	0.4	0.4	0.4	0.4	0.4	0.6	0.8	3.8	8.0	8.2	1.2	0.8	8.4	4.0	1.2	2.8	7.2
0.01 M. KCl	20.0	6.4	17.8	3.0	2.8	2.2	3.0	6.8	3.0	3.0	6.8	4.0	3.8	3.2	1.8	30.0	3.8	2.8	3.0	∞	3.8	2.8	5.0
1 M. sucrose	1.0	2.0	2.0	2.0	5.4	2.4	1.2	1.2	1.2	2.0	2.4	4.0	4.4	1.4	2.0	1.8	6.0	5.0	2.8	2.4	3.0	1.8	6.0
0.5 M. sucrose	14.0	3.4	2.2	5.0	3.0	1.8	2.4	2.0	52.0	4.6	2.2	5.4	2.0	3.6	4.0	3.2	2.2	1.8	1.6	3.2	2.4	2.4	6.4
H_2O, 20°C.	2.8	9.0	9.0	37.2	4.6	1.8	2.0	1.8	2.0	1.8	1.8	2.0	1.8	1.8	2.0	1.8	1.2	2.0	2.4	7.2	1.4	2.0	21.0
H_2O, 33°C.	2.4	4.2	5.0	9.0	1.0	1.4	5.8	1.8	1.8	2.0	1.8	2.2	1.2	1.8	1.2	1.2	1.4	2.2	2.0	2.0	2.0	2.0	1.8

These two types are easily contrasted if the rates $\left(\frac{100}{\text{Reaction-time}}\right)$ of the successive reactions are plotted in sequence (figs. 5 and 6). The

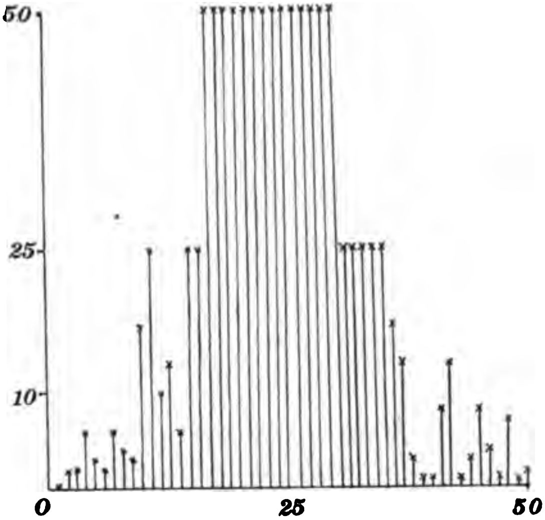


Fig. 5. Successive reactions of worms showing initial acceleration. This type of reaction is characteristic of the stronger concentrations of potassium salts. This graph is drawn from the first series in table 1 (0.1 molecular potassium chloride). The rates $\left(\frac{100}{\text{Reaction-time}}\right)$ are plotted as ordinates, and the sequence of reactions as abscissae.

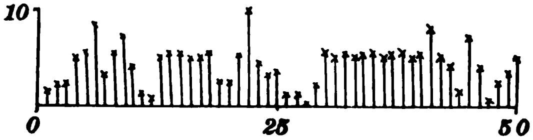


Fig. 6. Successive reactions of worms showing no initial acceleration. This type of reaction is characteristic of the weaker concentrations of potassium salts, of sucrose solutions and of water. This graph is drawn from the fifth series in table 1 (water 20°C.). The rates $\left(\frac{100}{\text{Reaction-time}}\right)$ are plotted as ordinates and the sequence of reactions as abscissae.

first type is illustrated in figure 5, which is a plotting of the rates of the successive reaction-times in the first series of table 1, viz., 0.1 molecular potassium chloride. The reaction is at first slow but after a few such slow reactions, acceleration takes place and the reactions become very rapid. An equilibrium is then established for a number of successive reactions, after which the slow rates gradually reappear. The second type is represented in figure 6, a plotting of the rates of the successive reaction-times in the fifth series in table 1, distilled water at 20°C. This type is illustrated not only by distilled water but also by the solutions of sucrose and the weaker solutions of salts. To these fluids the worms react more or less indifferently; at times they will remain in the solution for a considerable period, while in many cases they will withdraw from the liquid in less than three seconds; but there is never a definite acceleration in the reaction-time such as is seen in the first type. This irregularity in the reaction may be interpreted as meaning that these non-aquatic worms are being exposed to liquids of minimum stimulatory effect. In weaker solutions of salt, for example 0.01 molecular solution of potassium chloride (table 1), the worms make a complete entrance into the solution more frequently than is the case with distilled water.

Although in some series the experiments were carried as far as three hundred reactions, no acceleration took place, thus proving that at such a concentration the amount of reacting substance is insufficient to bring about the type of reaction characteristic of the stronger concentration of the salt solutions before the appearance of the muscular fatigue which would naturally prevent any further continuation of the experiment.

Furthermore the above experiments with sucrose and water show that osmotic pressure is not a significant factor in the sensory stimulation of the worms, since all yielded essentially the same result. Of much more importance is the fact that the solutions just mentioned show a marked difference in relation to the effect on the worms as compared with the salt solutions. What, then, is the cause of such a difference? Is the acceleration brought about by the direct combination of the receptive protoplasm with the salts or by the substance formed as a result of muscular activity? To test this, the worms were tried out under different temperatures. At 40°C. the worms reacted to distilled water by instant withdrawal as if it were a very strong solution of salt, for example, 0.5 molecular potassium chloride. A temperature was therefore chosen at which the worms did not react instantaneously but at which their muscular activities were considerably greater than at the temperatures in which the former experiments were carried out. On successive exposure of the worms to distilled water at 33°C. (table 1), while the room temperature was 20°C., no initial acceleration in the reaction-time took place, but a type of reaction similar to that brought about by the distilled water and sucrose solutions at 20°C. occurred. This indicates that the acceleration is not due to the products of muscular activity but must result from the specific action of the salt on the protoplasm of the sensory cells.

This conclusion is further supported by the following experiments. When a worm that had been stimulated by a salt solution to the point at which its reaction-time was shortest was transferred to a dish containing distilled water of the same temperature as that of the salt solution, its reactions were no longer nearly instantaneous, but were like those brought about by distilled water under ordinary conditions (table 2, worm A). But when the same worm was again transferred to the salt solution the very short reactions characteristic of the action of the salt was at once resumed. In this way the worm was transferred back and forth several times with the same result. On the other hand when a worm (table 2, worm B) was first exposed to distilled water for about twenty-five reactions and then transferred to the salt solution, the instantaneous reaction did not take place at once but appeared only after the worm had been dipping in and out of the salt solution for about twelve reactions. Thus it is clearly seen that the acceleration is brought about only by the action of the salt at proper concentration and not by distilled water. Furthermore, the reaction-time once accelerated by the action of the salt cannot persist except in the presence of the stimulating medium; when the salt solution is replaced by distilled water, the acceleration of the reaction at once disappears and is resumed only in presence of the salt solution.

When the same method of procedure was applied in the case of the weaker solutions of salt, for example, 0.01 molecular potassium chloride, into which the worms, as has already been mentioned, make complete entrance much more frequently than into distilled water, the difference in the reaction between such concentrations of salt and distilled water was clearly marked (table 2, worms C and D).

Thus it is made evident by the above experiments that sensory stimulation by potassium salts is due to the specific action of the salt, dependent essentially on the amount of the reacting substance present, and furthermore that such action may be distinguished from that of distilled water and sucrose solution.

The writer acknowledges her indebtedness to Dr. G. H. Parker, under whose supervision this work was carried out.

TABLE 3

Reaction-time in seconds of worms A, B, C and D to successive applications of pairs of stimulating fluids. Each pair consisted of water and some concentration of potassium chloride. Each worm was tested for a time first with one fluid, then with the other, and so on. The sign ∞ indicates that the worm entered the fluid instead of withdrawing from it

WORM		REACTION NUMBER																										
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
A	0.1 M. KCl..	∞	1.8	∞	1.0	∞	0.8	0.8	0.8													0.4	0.4	0.2	0.2	0.2		
	H_2O ..									1.0	1.0	4.8	2.8	1.8	3.2	3.2	1.8	1.0	1.0	1.8	1.4						0.1	14.2
B	H_2O	3.4	3 0	4 0	2.0	35.0	35.0	6.0	2.4	4.0	2.8	3.0	2.0	4.0	2.8	2.8	1.0	2.0	6.4	2.8	3.8	2.8	1.8					
	0.1 M. KCl..																							6.4	4.0	2.0	6.8	5.0
C	0.01 M. KCl.	∞	∞	1.8	2.0	60.0	4.0	4.2	8.0	3.4	10.0	5.0	16.4	5.8	∞	∞	6.8	4.8	3.2	6.2	2.8	6.8	∞	2.8	1.8	1.8	1.8	24.0
	H_2O ...																											
D	H_2O	12 0	5.0	15 0	3 0	4 0	6.4	2.2	12 0	15.0	5.0	3.0	7.0	4.0	5.4	3.4	18.0	79.0	1.8	5.4	5.0	8.4	30.0	8.2	30.0	15.0	2.8	1.8
	0.01 M. KCl.																											

WORM		REACTION NUMBER																										
		28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
A	0 1 M. KCl..																											
	H_2O ..	1.8	7.8	2 8	2.0	6.8	2.8	2.8	2.0	11.0	2.8	3.0	1.8	1.8	2.2	1.8	2.4	2.0	1.8	1.8	2.0	1.8	2.0	2.4	1.8	3.4	2.0	2.8
B	H_2O ..																5.4	3.8	1.8	2.0								
	0.1 M. KCl..	4 0	3.0	9.0	3 4	2.4	1.8	1.4	1.0	0.8	0.4	0.2	0.2	0.2	0.2	0.2					0.2	0.2						
C	0.01 M. KCl.	5.0	1 8	1 8	2.0	5.0	3.0	4.8	∞	3.8	3.0	16.0	∞															
	H_2O													1.8	1.8	2.0	1.8	2.0	1.4	1.8	2.0	1.8	3.2	2.0	1.8	2.0	5.2	5.0
D	H_2O	4 8	2 8									55.0	2.8	2.8	2.2	2.0	3.0	2.8	2.0	2.8								
	0.01 M. KCl.			70 0	1.8	42 8	4.8	∞	∞	∞	∞										1.8	2.0	3.0	∞	∞	1.8	∞	

WORM		REACTION NUMBER																									
		55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
A	0.1 M. KCl..		0.2	0.2					0.2												0.2	0.2	0.2				
	H_2O........	4.8			0.5	4.8	0.8	25.0		0.4	0.3	2.4	2.8	2.0	1.8	4.8	5.0	3.8	3.8	15.0				20.4	5.0		
B	H_2O........																										
	0.1 M. KCl..																										
C	0.01 M. KCl.			1.8	1.0	1.8	1.8	∞	6.2	∞	1.0	1.4	∞														
	H_2O........	2.2	5.4											4.8	5.0	1.8	1.2	5.0	3.8	7.8	3.0						
D	H_2O........	8.0	5.0	1.8	3.0	2.8	2.4	12.0	1.8	25.0	8.0	8.0	2.4													7.0	7.8
	0.01 M. KCl.													2.0	2.0	2.8	4.0	∞	1.8	2.0	∞	∞	2.0	2.8	∞		

SUMMARY

1. A new method for the determination of the effect of solutions on worms is described.

2. On comparing the efficiency of six potassium salts on the sensory cells of the dung-worm, Allolobophora foetida, it is found that the effect of the nitrate, chloride, acetate and sulphate is dependent on the amount of cations present, while in case of tartrate and citrate the amount of potassium ions is too small in all probability to attribute the effect to the cations.

3. On successive exposures of the worms to the potassium salt solutions of stronger concentrations (for example, 0.1 molecular potassium chloride), an initial acceleration in the reaction-time takes place. The reaction-times are at first long and become shorter and shorter until equilibrium is established, after which the reaction-time becomes long again.

4. On successive exposures of the worms to the weaker concentrations of potassium salt solutions (for example, 0.01 M. potassium chloride), to distilled water and to sucrose solutions, no initial acceleration in the reaction-times takes place.

5. When the worms in successive series are exposed to the distilled water at 33°C., while the room temperature is at 20°C., no acceleration in the reaction-time takes place, though the muscular activity of the worms increases considerably.

6. When a worm that has been stimulated by a salt solution to the point at which its reaction-time is shortest is transferred to a dish containing distilled water of the same temperature as that of the salt solution, its reactions are no longer nearly instantaneous, but are like those brought about by distilled water under ordinary conditions. But when the same worm is again transferred to the salt solution the very short reactions characteristic of the salt are at once resumed.

7. When the same method of procedure is applied in case of weaker concentrations of salt (for example, 0.01 molecular potassium chloride), into which the worms enter more frequently than into distilled water, the difference between the reactions in the salt solution and the distilled water is clearly marked.

8. The initial acceleration brought about on successive exposures of dung-worms to salt solutions is due to the specific action of the salts on the sensory cells.

BIBLIOGRAPHY

HAAS, A. R. '16. Science, n. s., xliv, 105.
PARKER, G. H., AND C. R. METCALF. '06. This Journal, xvii, 55.
SHOHL, A. T. '14. This Journal, xxxiv, 384.

THE SECRETORY PRESSURE OF THE LIVER WITH SPECIAL REFERENCE TO THE PRESENCE OR ABSENCE OF A GALL BLADDER

F. C. MANN AND J. P. FOSTER

From the Mayo Clinic, Rochester, Minnesota

Received for publication September 14, 1918

It has been determined that all of the extra hepatic ducts dilate after the removal of the gall bladder (1). This result seems to be due to interaction of the pressure exerted by the liver and the sphincter at the duodenal end of the common bile duct. It seemed desirable, therefore, to know whether the secretory pressure varied in species of animals with a gall badder from those without one. Our investigation is but one of several studies we have undertaken in reference to the function of the gall bladder.

The secretory pressure of the liver has been previously studied by several investigators. The earlier results obtained were quite variable. The most extensive and complete work on the subject was done by Herring and Simpson (2), who investigated both the secretory pressure of the liver and the process of bile absorption, after occlusion of the common bile duct. Their method of experimentation consisted in exposing the biliary tract with the animal under chloroform anesthesia and, after clamping the cystic duct, placing a cannula in the common bile duct. A vertical glass tube, having an internal diameter of 1 mm., was attached to the cannula. The lower end of the glass tube was adjusted so that the zero mark was at the level of the hepatic duct at the portal fissure. By means of a T-tube interposed between the cannula and the vertical tube, it was possible to record the rate of bile flow at zero pressure. Appropriate precautions were taken to keep the animal warm and in good condition. Many controls were taken in order to obtain trustworthy data. The maximum pressure to which the bile would rise in the tube was taken as a measure of the secretory pressure expressed in millimeters of bile. The pressure was measured in cats, dogs rabbits, guinea pigs and monkeys. Their results may be briefly tabulated as follows:

1. The average of 18 cats was 304.4 mm. bile; maximum pressure 375; minimum pressure 211.
2. The average of 8 dogs was 300.0 mm. bile; maximum pressure 342; minimum pressure 243.
3. The average of 6 rabbits was 259.5 mm. bile; maximum pressure 306; minimum pressure 181.
4. The pressure in two guinea pigs was 122 and 154 mm. bile, respectively.
5. The pressure in two monkeys was 220 and 321 mm. bile, respectively.

The secretory pressure of the liver was measured also in cats and dogs by Mitchell and Stifel (3). They found the average pressure of liver secretion in cats to be practically the same as that obtained by Herring and Simpson.

We measured the secretory pressure of the liver in anesthetized and unanesthetized animals, and since we wished to determine whether there was any relation between the secretory pressure of the liver and the presence or absence of the gall bladder, only comparable species were used.

The secretory pressure of the liver in anesthetized animals. Our method of measuring the secretory pressure of the liver in anesthetized animals differed in only a few respects from that used by Herring and Simpson. The animals which had been fasted for about twelve hours previously were lightly etherized, and a cannula was inserted into the common bile duct with its point directed toward the liver. In the smaller species the insertion of the cannula was often quite difficult. An upright glass tube with an internal diameter of about 2 mm. was attached to this cannula and arranged so that its lower end was roughly in the plane passing through the middle of the liver. Therefore the lower end of the tube marked practically zero pressure. The maximum distance that the bile rose in this tube, expressed in millimeters of bile, was taken as a measure of the secretory pressure of the liver. The operative work was always performed with as little trauma as possible and the general condition of the animals, as regards temperature, light anesthesia etc., was always well maintained. In some experiments the cystic duct was left patent while in others it was either ligated or a small clamp applied to it.

In the beginning of an experiment the bile usually would rise quite rapidly in the tube, but as the pressure increased the flow became slower and finally stopped. Quite frequently, after reaching a maximum height it dropped several millimeters before being maintained at a constant level. In some experiments the pressure was taken two or three times with an interval between. The maximum pressure, not including the respiratory rise, was always the one recorded.

The secretory pressure of the liver in animals having a gall bladder. The secretory pressure was measured by the method described in the species of animals having a gall bladder, as follows: guinea pig, rabbit, spermophile and goat.

Herring and Simpson report variable results in the guinea pig and the secretory pressure of the liver was rather low in their two successful experiments. Our results were fairly constant, and some of the pressures obtained were quite high (table 1).

Our results in the rabbit do not differ materially from those of previous investigators (table 1).

The secretory pressure of the spermophile (C. tridecemlineatus) was measured because it is fairly comparable in regard to size and habits with the rat, which does not possess a gall bladder. It was found to be quite difficult to obtain the pressure in this species probably because the liver was not able to secrete enough to fill the manometer with he animal under an anesthetic. We had many failures in this species. The pressure was found to be quite low (table 1).

The secretory pressure of the liver in the goat seems never before to have been measured In our experiments it was found to be relatively low (table 1).

The secretory pressure of the liver of species of animals which do not possess a gall bladder. Only two species of animals normally without a gall bladder—the rat and pocket gopher—were found suitable for experimentation. In both these species the pancreatic duct empties directly into the bile duct and consequently a combined pressure of the two glands was measured. However, by ligating the pancreatic duct before measuring the pressure it was determined in a few experiments that the effect of this gland was negligible. We attempted to determine the pressure in the horse but found it technically very difficult; the same was true of the pigeon.

The secretory pressure in the rat was easy to obtain and quite high relatively (table 1) It was found difficult to obtain the secretory pressure in the pocket gopher (G. bursarius), and all the pressures were relatively low (table 1).

Secretory pressure of the liver in unanesthetized animals. In order to determine how much the anesthesia affected the results, a few measure-

TABLE 1

Secretory pressure of the liver in various species of animals. Animals etherized in each instance. Two species, the rat and pocket gopher, do not possess a gall bladder

SPECIES	NO.	WEIGHT	CONDITION	
		grams		
Rabbits	1	2000	Good	308
	2	2440	Good	225
	3	2275	Good	245
	4	2155	Good	290
	5	1765	Thin	255
		Average		264.6
Guinea pigs	1	775	Good	200
	2	707	Good	210
	3	480	Good	190
	4	540	Good	195
	5	755	Good	218
		Average		202.6
White rats	1	190	Good	225
	2	160	Good	200
	3	180	Good	215
	4	190	Good	225
	5	165	Good	225
	6*	165	Good	195
	7*	160	Good	220
		Average		215
Pocket gopher	1	270	Good	140
	2	225	Good	125
	3	325	Good	160
	4	170	Good	130
	5	270	Good	180
		Average		147
Striped gopher	1	140	Good	125
	2	135	Good	125
		Average		125
Goat	1	24.0	Fair	185
	2	10.4 kg.	Fair	180
		Average		182.5

* Pancreatic duct ligated.

ments of the secretory pressure were taken in unanesthetized dogs. A small metal cannula was sutured into the gall bladder, practically eliminating the capacity of this viscus, and the common bile duct was doubly ligated and sectioned. After the animal recovered from the operative procedure, the secretory pressure was measured in the same manner as in the anesthetized animal and it was found to remain very constant in the same animal from day to day. In the sleeping dog the pressure usually varied between 320 and 345 mm. Under certain experimental conditions it would go quite high, in one instance going as high as 550 mm. From these results it seems that the secretory pressure is usually higher when the animal is not under an anesthetic. In none of our own experiments nor in those recorded has the secretory pressure of the anesthetized dog been noted to be, as an average, quite so high as in most instances in which it was measured in the unanesthetized animal.

CONCLUSIONS

The secretory pressure of the liver was found to vary considerably in the various species of animals. The reason for this is not clear; there may be many causes; however, the presence or absence of the gall bladder does not seem to be one of them. The secretory pressure of the liver appears to be somewhat greater in unanesthetized animals than in those under an anesthetic but since the data obtained on anesthetized animals were only comparative, the conclusion that the presence or absence of the gall bladder bears no relation to the secretory pressure of the liver is justified.

BIBLIOGRAPHY

(1) JUDD AND MANN: Surg., Gynec. and Obst., 1917, xxiv, 437.
(2) HERRING AND SIMPSON: Proc. Roy. Soc., London, 1907, lxxix, 517.
(3) MITCHELL AND STIFEL: Johns Hopkins Hosp. Bull., 1916, xxvii, 78.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 DECEMBER 1, 1918 No. 3

CHEMICAL STIMULATION OF THE CEREBRAL CORTEX

S. S. MAXWELL

From the Rudolph Spreckels Physiological Laboratory of the University of California

Received for publication September 19, 1918

Some years ago I pointed out that a broad distinction may be drawn between two classes of brain stimulants. The one class, which includes compounds like barium chloride and the calcium precipitants well known to stimulate medullated nerves, acts only on the white matter (1). These substances have no direct stimulating effect on the gray matter although Sabbatani (2) and others have shown that their presence may increase the irritability of the cortex to electrical stimulation.

The other class of chemical stimulants includes such substances as creatin, strychnine and picrotoxin. Landois (3) first pointed out that creatin and creatinin can excite the motor areas when applied directly to the exposed surface of the cerebral hemispheres. Baglioni and Magnini (4) showed later that strychnine used in the same way is a powerful excitant. On the other hand I was able to show that creatin acts on the gray matter only, since it has no effect on the white matter or on medullated nerve (5). The action of strychnine like that of creatin is confined to the gray matter.

The different effects of these two classes of chemical stimulants might be due to differences in permeability of the tissues or to differences in chemical constitution of the irritable elements, or both these factors might be involved. It is certainly fair to assume that the permeability of nerve cells and relatively naked dendrons must differ from that of medullated nerve. On the other hand the work of Moore (6) on chemical differentiation in the nervous system indicates that nerve cells themselves differ chemically. In proof of this Moore apparently has used only substances which would fall into my second class.

Since the contrast between these two classes of compounds in their stimulating action on the nervous system is so marked I was interested to discover any substance which could act in both ways. The remarkable fact that ammonium chloride does not stimulate medullated nerve while its substitution product, tetraethyl-ammonium chloride, was found by Loeb and Ewald (7) to be even more effective than sodium citrate, led me to try the latter substance on the cortex of the motor areas. I found indeed that tetraethyl-ammonium chloride is a more powerful stimulant of gray matter than creatin though less powerful than strychnine.

All my experiments were performed on rabbits. The brain surface in the region of the motor areas was laid bare by trephining, the dura was carefully removed and the localization verified by electrical stimulation. A small crystal or a small quantity of the powdered tetraethyl-ammonium chloride was then applied to the exposed surface. After a latent period of five to ten minutes muscular twitchings began in the same body regions which had responded to electrical stimulation of the cortex. The contractions gradually increased in intensity to a maximum which was usually reached in twenty or thirty minutes. The latent period was noticeably shorter and the excitation greater than when creatin was used. In order to make a more exact comparison, symmetrically situated cortical areas were exposed; powdered creatin was applied to one side and tetraethyl-ammonium chloride to the other. Without any exception the contractions resulting from the action of the tetraethyl-ammonium chloride preceded by several minutes those caused by the creatin on the opposite side.

By the method just described the stimulating effect of tetraethyl-ammonium chloride was also compared with that of strychnine. In every instance a 1 per cent solution of strychnine applied to the cortex on one side brought about the characteristic twitchings and convulsive contractions more promptly than the concentrated solution of tetraethyl-ammonium chloride applied to the other side. In a few experiments comparisons were made between tetraethyl-ammonium chloride and sodium citrate or sodium oxalate.. In none of these was there any indication that the citrate or oxalate had a stimulating effect.

By the above experiments it is shown that tetraethyl-ammonium chloride unlike the other substances which stimulate medullated nerve is also a stimulant of gray matter. I hope to point out in connection with a report on other experiments the bearing of these facts on theories as to the nature of chemical stimulation.

BIBLIOGRAPHY

(1) **Maxwell**: Journ. Biol. Chem., 1906, ii, 183.
(2) **Sabbatani**: Arch. ital. d. Biol., 1905, xliv, 398.
(3) **Landois**: Deutsch. med. Wochenschr., xiii, 685.
(4) **Baglioni and Magnini**: Arch. d. Fisiol., 1909, vi, 240.
(5) **Maxwell**: Journ. Biol. Chem., 1907, iii, 21.
(6) **Moore**: Proc. Nat. Acad. Sci., 1917, iii, 598.
(7) **Loeb and Ewald**: Journ. Biol. Chem., 1916, xxv, 377.

THE BLOOD-PRESSURE CURVE FOLLOWING AN INTRASPINAL INJECTION OF ADRENALIN

J. AUER and S. J. MELTZER

From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research

Received for publication September 30, 1918

The discovery by Oliver and Schäfer that adrenal extract causes a rise of blood pressure was made by administering the extract intravenously. The blood-pressure curve which follows such an injection is characteristic. Practically without a latent period, the pressure curve rises and reaches its maximum height in a very short time. There is almost no plateau; the descent is gradual; the original pressure is attained in about five to seven minutes. Sometimes the pressure falls for a short period below the original level.

We studied at various times the character of the pressure curve which follows after diverse methods of administration of adrenalin. Among others, we studied the pressure curve which follows intraspinal injection of adrenalin in monkeys under the generally accepted supposition that absorption from the spinal canal is a slow process. The results were quite interesting. In most instances the blood-pressure curve was characteristic; the latent period was prolonged, the rise was comparatively slow, the maximum, although quite high, was only attained after several minutes and continued plateau-like for a fairly long time; the descent to normal was, comparatively speaking, very slow. These findings were embodied in a brief communication before the Society for Experimental Biology and Medicine (1).

About simultaneously with our report there appeared a brief communication of Dixon and Halliburton on the rapidity of the absorption of drugs into the cerebro-spinal fluid (2). There they stated that

> A small dose of adrenalin (1 cc. of 1 in 10,000) solution, will equally rapidly produce a typical rise of arterial blood pressure, in fact almost as high as if it were injected intravenously.

In their experiments the drug was introduced through a cannula inserted into the subcerebellar cisterna. In a later article however, Dixon and Halliburton (3) make the following statement:

> Since the publication of our preliminary note, Auer and Meltzer have made some intraspinal injections of adrenalin which they expected would have no stronger action on blood pressure than that of a subcutaneous injection. They employed monkeys and injected large doses; the arterial pressure rose slowly and reached its maximum in a few minutes and remained high for a considerable time. The injections were made into the lumbar region. We have made some injections into the lumbar region but there, as we have already stated, absorption is comparatively slow and several minutes are required before any noticeable effect is observed.

Thus there is no contradiction between our observations and the results obtained by Dixon and Halliburton. In the last mentioned communication they rather confirmed our observations that when the injection of adrenalin is given in the lumbar region the absorption is comparatively slow, and several minutes are required before any noticeable effect is observed.

Dixon and Halliburton, however, fail to make any statement regarding the final intensity or the long duration of the effect. These authors were apparently interested mostly in that part of our communication which seems to contradict their statement, namely, that the absorption of adrenalin from the cerebro-spinal fluid is almost as rapid as if it were injected intravenously and confined themselves to the explanation of the seeming contradiction by the fact that our experiments were made by injections into the lumbar region while they made their injections into the subcerebellar cisterna. They were not interested in the details of the effect of intralumbar injections.

To us it seems that the most interesting part of our observations consists in the facts that the maximum of the blood-pressure rise following an intraspinal injection of adrenalin has a comparatively long plateau and the descent of the curve to the normal level is very slow; in other words, that the blood-pressure rise following an intraspinal injection of adrenalin is as a rule the longest ever observed after a single injection of adrenalin. This fact is interesting from a theoretical point of view; but it may, as we have pointed out elsewhere (4) have an important practical application. We intend therefore to describe the results of our experiments in some detail and, especially, to reproduce a most striking curve to illustrate the effects of an intraspinal injection of adrenalin. The present high price of monkeys prohibits a repetition of our experiments on a large scale, but through the courtesy of Doctor Flexner we were enabled some time ago to make a few observations on three monkeys. These observations will be included in the following general statement.

EXPERIMENTAL

Except in monkeys and human beings, it is impossible in other animals for an intraspinal injection in the lumbar region to enter the subarachnoidean space of the canal below the spinal cord; in most cases the needle enters into the cord itself and the injection is made into its tissue. In monkeys an injection below the spinal cord is frequently possible when the needle is inserted between the third and fourth or between the fourth and fifth lumbar vertebrae. However, even in monkeys the injection is not successful in every case. We have learned in the experiments with adrenalin as well as in our extensive experience with magnesium sulphate that when the escape of spinal fluid through the needle is very scant, the effect of an injection is frequently poor and not characteristic. Furthermore, on account of a venous plexus on the ventral surface of the canal and the minuteness of the cavity of the subarachnoidean space below the cord, the hypodermic needle sometimes enters into the venous plexus and the result of the injection is either that of a mere intravenous injection or, more frequently, of a mixture of an intravenous and an intraspinal injection; in the latter case the injected quantity is too small to produce a characteristic effect of an intravenous as well as of an intraspinal administration. In human beings the condition is apparently much more favorable. In the numerous injections of adrenalin made intravenously in children in the course of the epidemic of poliomyelitis (5) the effect of adrenalin upon the region of the cervical cord was unmistakable whether the quantity injected amounted only to 1 cc. or to 6 cc. for the small children afflicted with the disease.

Our experiments with the lumbar injection of adrenalin were made on ten small monkeys (macacus rhesus). The injections were as a rule made in the spaces between third and fourth or fourth and fifth lumbar vertebrae. In a few instances the injection was given in the dorsal region—with an effect generally characteristic for injections in the lumbar region. Each animal was used for several experiments after intervals of hours or several days. The smallest dose employed was 0.5 cc. and the largest 1.5 cc. of a 1: 1000 solution of adrenalin.

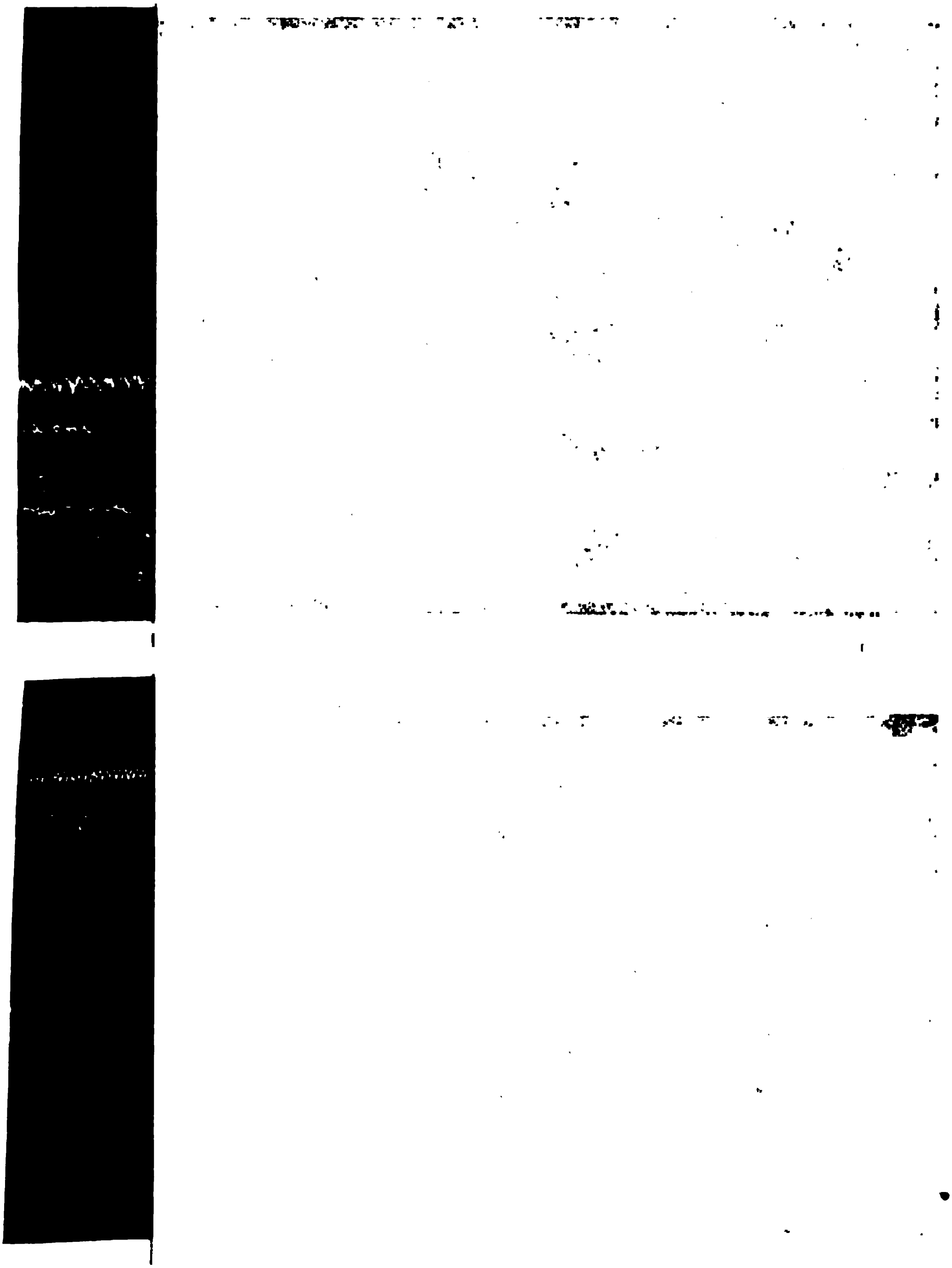
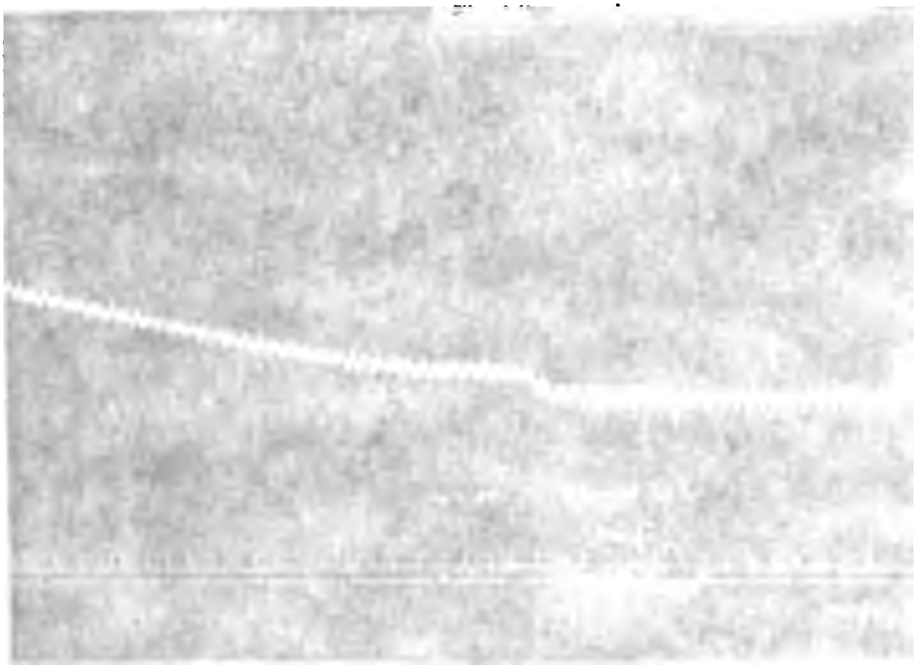


Fig. 1. The
pressure, the
jection of adr
it was 140 mm
continued for
pressure was
and 3 minutes
the original bl



The character of figure 1 reproduced in this paper is typical for most of our results although we have to state that it represents the most striking result which we have obtained in our series of experiments. It was obtained January 17, 1912, from a second injection of 1.5 cc. adrenalin into the lumbar region of a male monkey; no spinal fluid was obtained at this (second) lumbar puncture. The original blood pressure was 62 mm. mercury. There was no initial drop of blood pressure following the injection, and the rise began after a latent period of eight seconds. The maximum height of 172 mm. was reached in six minutes. The maximum level presented practically a plateau of several minutes duration. The pressure fell exceedingly slowly; for one hour after the injection the blood pressure was still 48 mm. of mercury above the original level (see figs. 1 A, 1 B, 1 C and 1 D).

Figure 2 is from an intravenous injection of 1 cc. of adrenalin administered to a male monkey (2260 grams) November 8, 1917. This tracing is reproduced here for the purpose of emphasizing the contrast between intraspinal and intravenous injections of adrenalin. In this experiment the original pressure was 40 mm. mercury. There was no drop or obvious latent period preceding the beginning of the blood-pressure rise; in eight seconds the maximum of 148 mm. mercury was reached; there was no plateau. After eight minutes the blood pressure was again 47 mm. mercury, a level only slightly higher than the original blood pressure.

Without entering upon the details of our experiments, we may make the following general statement. With negligible exceptions, we may say that the blood-pressure elevation brought about by an intraspinal injection is characterized by a slow rise, by a "plateau-like" perseverance of the maximum rise, by a comparatively very slow fall to the normal level and, in general, by a long duration of the pressor effect when compared with an intravenous injection. These blood-pressure curves vary with the individual animal and its health; with the presence of fluid in the spinal cavity below the cord; with the quantity of adrenalin injected and to a degree also with the [illegible] injections of adrenalin administered to the same animal [illegible] of the interval between the injections, and especially [illegible] of injection, for a lumbar injection is distinctly more [illegible] thoracic one.

In some instanc[illegible] first effect of a[illegible] [illegible]ction was an initial dr[illegible] [illegible]sure. The d[illegible] [illegible] of short duration [illegible] [illegible]ically in [illegible] [illegible]aracteristic

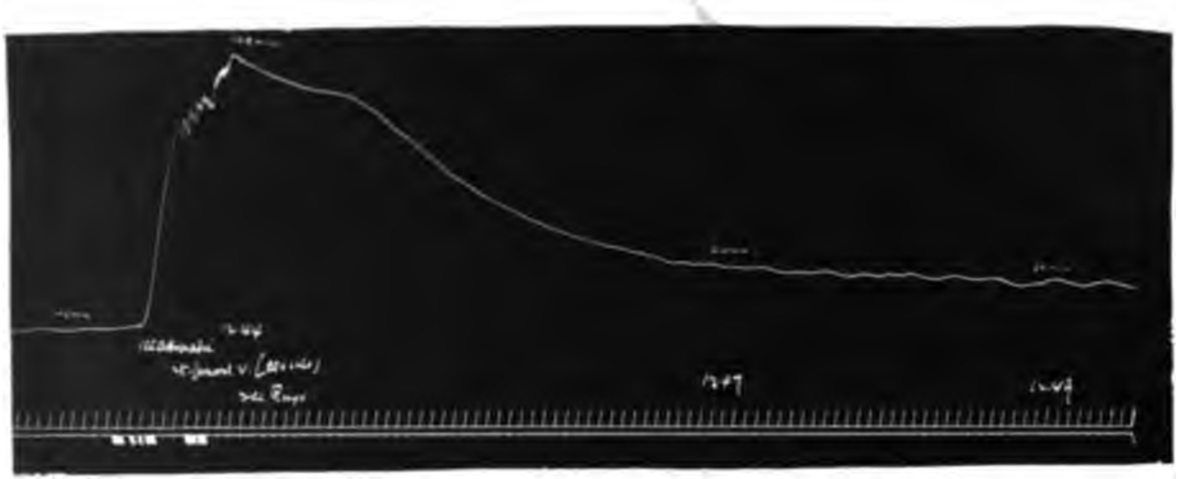


Fig. 2. Shows the blood-pressure curve after an *intravenous* injection of 1 cc. adrenalin into a monkey. The first line represents the pressure curve (pulse pressure very small), the second carries the time markings (4 seconds) and marks the base line while the third line shows the time of the injection of adrenalin and the washing down of adrenalin by 2 cc. of a Ringer solution. The blood pressure before the injection was only 40 mm. The injection of 1 cc. of adrenalin into the right femoral vein raised the blood pressure abruptly and the immediate injection of 2 cc. of Ringer solution, to wash out the adrenalin remaining in the cannula, brought the blood pressure to its maximum height of 148 mm.; the maximum was thus attained 20 seconds after the injection of adrenalin. About 5 minutes later the pressure fell to 64 mm. and gradually came down to a level which in this case remained slightly above the original low blood pressure.

rise. In one case after a lumbar injection of 1.5 cc. the drop was as great as 52 mm. mercury, the pressure falling from 116 to 64 mm., but it soon began to rise and in four minutes reached a height of 162 mm. mercury. In one animal however, after an injection of 1 cc. (no spinal fluid) the blood pressure slowly fell 10 mm. with no subsequent rise of pressure. This monkey was sick. However, after a second injection of 1 cc. adrenalin into the same monkey, no drop of blood pressure occurred and after a latent interval of forty seconds the pressure rose in eight minutes from 100 mm. to 138 mm.; this rise lasted twenty-two minutes. In these two injections the puncture yielded no spinal fluid. Later, in the same animal, after an injection of 1.5 cc. adrenalin, there was first a short preliminary rise followed by a drop to 86 mm. mercury lasting twenty-four seconds and then, during two minutes, the blood pressure rose to a maximum of 143 mm.; after forty-seven minutes the pressure sank again to 84 mm. In this instance the puncture had shown the presence of spinal fluid. In another animal an injection of 1 cc. caused a drop from 72 to 48 mm., that is, a drop in blood pressure of 24 mm. mercury which was not followed by any rise. However, twenty-eight minutes later, after a second injection of 1 cc. there was neither a drop of blood pressure nor was there any latent period, but in the course of three minutes after this injection the blood pressure rose from 50 mm. to 124 mm. of mercury. (Study of the further course of blood pressure in this experiment was prevented by the formation of arterial clots.)

The preceding results were observed after injections into the lumbar region. We have, however, records of a few observations on the effects of intraspinal injections when the site of application was the thoracic region of the cord. In one instance, after an injection of 1.5 cc. of adrenalin into the fifth thoracic interspace, there was no initial drop of blood pressure; a latent period of thirty-six seconds elapsed before the rise began and in two minutes the pressure rose from 54 mm. mercury to 196 mm.; after twenty-four minutes the pressure was still 100 mm. of mercury. In another animal an injection of 1 cc. of adrenalin in the fifth thoracic vertebral interspace brought an initial drop of blood pressure of 25 mm. which lasted two minutes. Then in the course of the following five minutes the blood pressure rose from 102 mm. to a maximum of 170 mm. and after fifteen minutes the blood pressure was still 134 mm. mercury. In general, however, the rise of blood pressure after intrathoracic injections of adrenalin was neither very high nor of striking duration.

CONCLUSIONS

Intraspinal injections of 1 cc. or 1.5 cc. of adrenalin in the lumbar region of monkeys cause a rise of blood pressure distinctly different in character from the curve obtained after the intravenous injection of adrenalin. The curve of blood pressure following an intraspinal injection is generally characterized by a slow rise from the original level to the maximum height; by a plateau-like duration of the maximum; by a slow fall to the blood pressure which prevailed before the injection of adrenalin. It may be stated in general that an intralumbar injection of adrenalin in monkeys causes a much more lasting effect upon the rise of blood pressure than an intravenous injection. It may perhaps be added that, as a rule, after intraspinal injections the pressure at the end of the pressor effect has not been observed to fall below the original level.

The question discussed in this paper has not been studied in human beings. While there is sufficient experience to show that an intralumbar injection of adrenalin in human beings is apparently innocuous, the effect of such an injection upon the blood pressure has not been studied properly. When we consider, as we stated before, that in human beings an intralumbar injection can be carried out with greater safety and certainty than in monkeys, the advisability of such studies in clinical physiology is quite evident.

BIBLIOGRAPHY

(1) Auer and Meltzer: Proc. Soc. Exper. Biol. and Med., 1912, ix, 79.
(2) Dixon and Halliburton: Journ. Physiol., 1912, xliv, 7.
(3) Dixon and Halliburton: Journ. Physiol., 1916, i, 198.
(4) Auer and Meltzer: Journ. Amer. Med. Assoc., 1918, lxx, 70.
(5) See W. L. Carr's paper on Poliomyelitis read at the Amer. Ped. Soc. (Arch. Ped., 1917, August); also Haas: Med. Rec., 1916, xc, 425.

XVII. THE METABOLIC GRADIENT UNDERLYING COLONIC PERISTALSIS

WALTER C. ALVAREZ AND ESTHER STARKWEATHER

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication October 2, 1918

In previous studies on the stomach (1), small intestine (2) and cecum (3), we have found that there is a gradient in the CO_2 output and catalase content of the muscle from one end of the organ to the other. We came to the conclusion that the metabolic gradient thus discovered underlies and probably gives rise to the gradients of irritability, rhythmicity and latent period which in their turn probably determine the direction of peristalsis in the tube. In this paper we wish to report similar studies made upon the colon. It should be stated here for the benefit of those who may not have read the previous papers that we found the curves of catalase content and CO_2 output so nearly parallel that we feel that the catalase is a pretty good index to the metabolism of the tissue. When confronted at first by the great variations in the figures obtained in different animals of the same species we rather despaired of being able to use what seemed to be so inaccurate a method. The remarkable constancy, however, of the gradation in any one intestine has satisfied us that these determinations are trustworthy.

Technic. The method used has already been described (2). Briefly, we have made five determinations at a time, using a shaking machine. We have used rats, guinea pigs, rabbits, cats and dogs. These animals were killed by a blow on the head or by chloroform and bleeding. The segments were removed immediately. In cats and dogs the muscle could be peeled off from the mucosa but in the smaller animals the mucosa had to be scraped off. In all the tables the figures represent cubic centimeters of oxygen at room temperature and atmospheric pressure, liberated in fifteen minutes from 30 cc. of 1.5 per cent neutral hydrogen peroxid by 0.3 gm. of finely minced muscle. The figures 1 to 5 on the left hand side represent segments taken at intervals along the colon from the ileocecal sphincter to the anus.

Cat colon

																							Average
1. Upper	45 1	23 5	12 8	31.3	43.3	38 2	45 0	30.0	31.4	39.7	30.0	34.6	29.3	21.0	32.8	27.8	33.0	48.6	28.5	29.9	33.5	35.6	29.5	32.8
2.	42 3	17 9	10 0	29.1	27.5	35.1	36.5	29.9	21.4	41.5	23.5	24.0	23.1	18.9	27.8	18.1	29.0	40.0	24.6	20.4	20.3	19.1	27.8	26.5
3.	38 5	12 5	9 5	29.2	26.2	35.3	37.3	26.9	21.1	30.2	22.4	24 2	22.6	24.4	26.3	21.4	19.1	31.6	22.8	18.5	23.8	28.6	22.2	25.0
4.	29 6	12 7	9.8	32.8	31.5	40.2	43.2	29.3	21.4	31.7	24 6	29.0	29.4	25.1	17.1	19.2	18.8		26.0	19.5	27.1	29.7	22.3	25.9
5. Lower	31 1	12.3	8 4	31.8	26.1	34.5	39.1	14.5	17.3	35.1	23.0	28.1	28 0	20.8	17.6	24.7		28.0	35.6	27.3	23.4	23.2	26.7	25.4

Dog colon

												Average
1. Upper	17.9	21.1	22.2	20.2	18.0	16.5	16.6	21.6	16.4	21.2	18.6	19.1
2.	17.5	17.5	11.1	13.9	13.3	15.7	16.1	18.8	13.4	20.0	17.8	15.9
3.	15.5	15.2	16.8	17.7	14.2	15.3	12.1	14.4	14.1	17.3	17.3	15.5
4.	14.6	16.2	11.8	13.3	6.7	16.7	12.2	12.0	15.1	16.9	16.9	13.9
5. Lower	15.1	14.8	7.1	6.7	14.1	12.1	11.7	8.5	11.5	13.6	16.2	11.9

Guinea pig colon

							Average
1. Upper	47.0	47.8	45.4	46.8	51.0	48.5	47.8
2	39.2	35.0	38.7	27.6	50.9	48.8	40.0
3	44.5	43.6	31.9	29.4	40.7	31.2	36.9
4	31.8	37.8	31.5	25.3	26.5	24.5	29.6
5. Lower	37.2	37.3	26.8	18.7	20.3	27.4	27.9

Rabbit colon

									Average
1. Upper	40.0	44.6	41.4	28.4	27.9	26.4	26.0	25.0	32.3
2	33.5	31.5	37.8	23.4	22.0	21.0	18.4	20.0	25.9
3	21.7	33.7	29.6	24.7	22.5	20.9	17.8	18.6	24.1
4	27.4	33.4	30.0	20.0	21.0	20.3	18.5	23.2	24.2
5. Lower	18.6	28.4	26.2	18.7	17.6	18.8	13.0	22.8	20.5

White rat colon

							Average
1. Upper	21.4	18.6	19.4	24.3	37.0	21.1	23.9
2	20.1	18.5	20.0	20.0	35.7	20.4	22.4
3	19.7	17.4	19.8	20.0	37.3	14.7	21.5
4. Lower	12.2	12.2	8.3	11.0	23.3	12.9	13.4

Ileo-cecal junction. Some observations by Alvarez (4) suggested that the ileocecal region in rabbits has a higher rate of contraction than that of the ileum a little above. Keith (5) feels that this is an important point in connection with his discovery of what seems to be a band of nodal tissue in that region. Unfortunately we have been unable to study the catalase content of the muscle in the sacculus rotundus of the rabbit because it cannot be separated from the mass of lymphoid tissue at that point. As would be expected, this tissue has a high catalase content. The following figures from the rabbit show no definite increase of catalase in the segment next to the sacculus. In the cat and dog where pure muscle could be obtained, there was nothing peculiar about the catalase content of the terminal ileum and ileocolic sphincter.

	CAT (MUSCLE) AVERAGE OF THREE	DOG (MUSCLE) AVERAGE OF TWO
1. Ileum	29.7	19.9
2. Ileum	30.4	19.7
3. Terminal ileum	27.3	18.4
4. Sphincter	26.5	15.9
5. Colon	23.9	14.0

	RABBIT (MUSCLE AND MUCOSA) AVERAGE OF SEVEN
1. Ileum	43.9
2. Ileum	42.5
3. Terminal ileum	42.9
4. Sacculus	56.1
5. Sacculus	56.9

CO_2 estimation. Although our previous work has made us feel that we can accept the catalase content as a fair index of the metabolic rate, we thought it best at the same time to make a few estimates of the CO_2 production. We have used Haas' method, which was described fully in a previous paper (2). Briefly, weighed segments of washed gut are sealed into small test tubes containing warm Locke's solution to which a little phenolsulphonephthalein is added. The CO_2 formed in the tissue gradually acidifies and decolorizes the solution. After this decolorization has proceeded sufficiently, the tubes are graded according to color, the palest being on the left. In the following tables the figures represent parts of the colon from cecum to anus—1 being near the ileo-cecal sphincter and 5 near the anus. Sometimes two or three tubes were so nearly of the same color that they have been bracketed together.

Cat	*Rat*
$\overbrace{3\quad 4}\quad 5\quad 2\quad 1$	$2\quad 1\quad 3\quad 4$
$3\quad 1\quad 2\quad 5\quad 4$	$1\quad 2\quad 3\quad 4$
$5\quad 3\quad \overbrace{1\quad 2\quad 4}$	$1\quad \overbrace{2\quad 3}\quad 4$
$5\quad \overbrace{1\quad 2}\quad \overbrace{3\quad 4}$	$2\quad 1\quad 3\quad 4$
$1\quad 2\quad 3\quad 5\quad 4$	$3\quad 2\quad 4\quad 1$
$2\quad \overbrace{4\quad 5}\quad \overbrace{3\quad 1}$	$2\quad 1\quad \overbrace{3\quad 4}$
$3\quad 1\quad 2\quad \overbrace{4\quad 5}$	$2\quad 1\quad 3\quad 4$
$1\quad \overbrace{2\quad 5}\quad 3\quad 4$	$1\quad 2\quad 4\quad 3$
$1\quad 2\quad 5\quad 3\quad 4$	$2\quad 1\quad 3\quad 4$

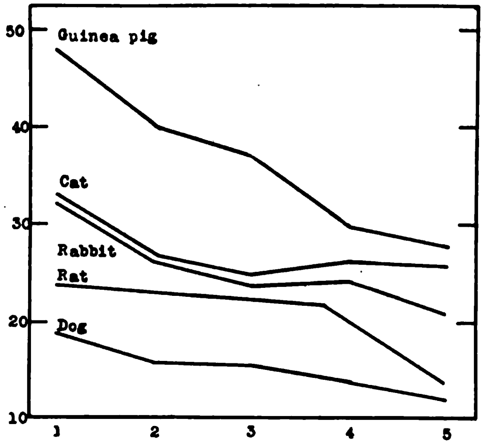


Fig. 1. Average gradient of catalase content of the colonic muscle in various animals. The ordinates represent catalase values expressed in cubic centimeters of oxygen liberated; the abscissae represent the five segments from which muscle was taken.

In some instances the gradation was brought out by titrating the solution back to its original color. The following figures represent the average number of drops of n/50 NaOH required for the different segments.

Cat

5.4 5.1 5.4 4.4 4.9

Rat

5.5 4.8 4.0 3.1

DISCUSSION

Here again in the colon we find gradients of catalase content and CO_2 production similar to those in the stomach and small intestine. When the catalase averages are plotted as in figure 1, it is seen that the gradient is most pronounced and most even in the guinea pig, rabbit and rat. It is less pronounced in the dog; and in the cat the aboral third is actually reversed. The individual curves in a number of cats were even more irregular, as can be seen in figure 2. There the only fairly constant features are the drop from the cecum to segment 2 and the rise from segment 4 to segment 5. Many of the dogs showed a similar type of curve but the average yielded a nearly straight line with a slight gradient downward to the anus.

The CO_2 estimations in the cat and rat showed gradations corresponding fairly well to those observed with the catalase in those animals. Attempts to show a gradation in rhythmicity down the colon have not been entirely successful on account of the irregular character of the contractions in excised segments. In the rabbit, however, the average rate seemed definitely higher in the upper part of the colon than in the lower.

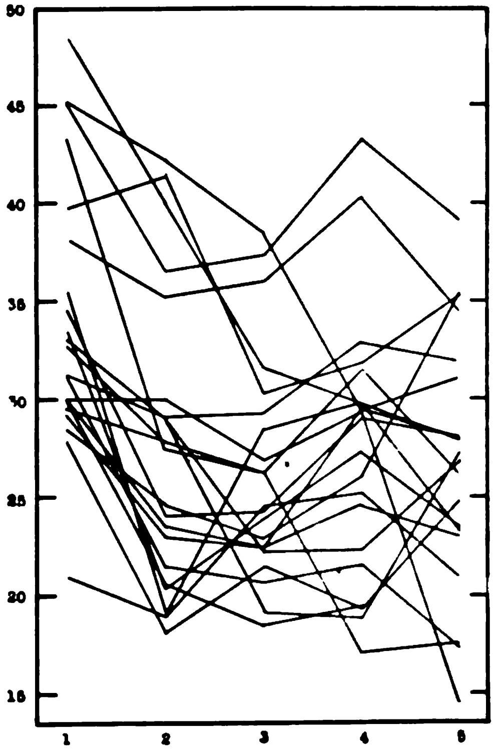


Fig. 2. To show the variations in the gradient of catalase content in the colons of individual cats. The ordinates represent catalase values and the abscissae segments along the colon.

Before proceeding to a discussion of the possible significance of these findings it might first be well to review briefly what is known about colonic peristalsis. In the first place the movements are more sluggish than those of the small bowel and the neuro-muscular apparatus seems less sensitive to stimuli. Thus one of us found it almost impossible to get any records of the colonic contractions in a woman with a fistula into the descending colon. The stimulus of blowing up the balloon sometimes gave rise to one or two contractions but after that even alternating deflation and inflation had little or no effect. Things were very different in several men with jejunal fistulae. In them the bowel was constantly contracting and pulling upon the balloon.

In man there are probably only a few large propulsive movements in the colon during the day. These occur usually after meals and often bring about a desire for defecation. The colons of rabbits, cats and dogs exposed under salt solution also remain quiet for long periods of time. We would expect then to find that the colonic neuro-muscular apparatus is different from that in the small intestine and more like that in the cecum (3). The differences that we have found in the rhythmicity, type of contraction curve, latent period and reaction to drugs (6) have all suggested that the metabolism is more sluggish in the colonic wall than it is in the wall of the small bowel. We were not surprised therefore to find the catalase content and the CO_2 output low in the colon. In the cat and dog the catalase content of the first colonic segment was often higher than that of the last ileal segment but the rest of the colon showed values lower than the lowest in the small bowel. The CO_2 output of the colon was generally much less than that of the lower ileum. A slight increase in metabolism on the lower side of the ileo-cecal sphincter might have something to do with the physiological stasis in the terminal ileum.

The striking peculiarity about peristalsis in the colon is that it is often reversed, particularly in the proximal half. Cannon (7) observed this first in the cat where the phenomenon is perhaps most marked. It has been seen in the dog, rabbit, rat, guinea pig, hedgehog and ferret (8). Retrograde transport of material in the human colon is commonly seen but the actual reverse waves are hard to demonstrate. Case (9), however, reports having seen them in 37 out of 1500 cases. As was to be expected, they were most pronounced in the presence of colonic obstruction or with spastic constipation accompanied by or due to an increased tone at the lower end of the bowel.

In view of this tendency to anastalsis it is not surprising to find that the gradient in the colon is irregular and reversed in places. In the small bowel, where katastalsis is the rule, the gradient was steep and even in all the animals. It is somewhat disappointing, however, to find in the cat the steepest and most constantly downward part of the catalase gradient on the right side near the cecum, just at the point where the reversed waves are most commonly seen.

Another interesting thing is the fact that in the herbivorous guinea pig and rabbit the gradient was more constant, more even and steeper than in the carnivorous cat and dog. We have suggested in a previous paper that it may be that the herbivora, with their rough bulky food must maintain a better gradient in their digestive tracts than is needed by the carnivora with their more concentrated, easily liquefied food and their short powerfully-muscled intestines.

Another point of interest is the rise in the gradient at the lower end of the cat's colon. Similar rises were observed in a number of the dogs. They were not observed in the rabbit, guinea pig and rat. It is suggestive that the first two animals defecate at comparatively rare intervals much as man does. They can thus comport themselves properly as pets in houses and they often seem to be quite constipated. The other animals, with the steep gradient down to the anus, defecate at short intervals, apparently as often as feces reach the rectum. A number of writers have claimed that in man there is a rise of tone up to the sigmoid or rectum (10), and one of us suggested several years ago that an increase in the steepness of the gradient up to the anus might be one of the principal factors in producing constipation (11). So far we have been able to get fresh segments from but one human colon. The cadaver was that of a man who died from a wide-spread terminal infection following a jejunostomy for an inoperable carcinoma of the stomach. The figures were as follows:

Cecum	*Colon*			*Rectum*
1	2	3	4	5
19.3	31.3	30.6	22.0	22.3

One cannot draw many conclusions from one case and a diseased one at that. There is, however, a definite downward gradient from the ascending colon to the rectum with perhaps a slight rise up to the anus. The low figure for the cecum, if normal for man, would account for the retention of fecal material in that region.

SUMMARY

Previous work on the small intestine and stomach showed gradients of catalase content and CO_2 production which the writers believe underlie and give rise to the gradients of rhythmicity, irritability and latent period which determine the direction of peristalsis.

Similar gradients are now shown to exist in the colon. The gradient of catalase content is steeper and more regular in the herbivorous rabbit and guinea pig than in the carnivorous cat and dog. The omnivorous rat has a fairly even gradient which is steep at the anal end. It is suggested that the herbivora with their rough bulky food and their thin-walled bowels need a more even gradient than the carnivora do.

In the cat and dog the lower end of the gradient is often reversed, that is, uphill towards the rectum and anus. It is suggested that this reversal saves these animals from frequent calls to defecation. The rabbit, guinea pig and rat defecate at short intervals, probably whenever material reaches the anus; the cat and dog empty their bowels more as man does and they sometimes are constipated. It is suggested that constipation may be due to an accentuation of the reversed gradient up to the anus. It is also suggested that the uneven gradient in the colon is associated with the well-known reverse peristalsis in that part of the gut. The more sluggish metabolism of the colonic muscle as compared with that of the small intestine was to be expected. No peculiarity could be observed in the catalase content of the ileocecal sphincter.

In one human case studied the gradient was quite similar to that in the dog, with the exception that the cecal figure was very low.

BIBLIOGRAPHY

(1) ALVAREZ AND STARKWEATHER: This Journal, 1918, xlvii, 60.
(2) ALVAREZ AND STARKWEATHER: Ibid., 1918, xlvi, 186.
(3) ALVAREZ AND STARKWEATHER: Ibid., 1918, xlvi, 563.
(4) ALVAREZ: Ibid., 1914, xxxv, 184; 1915, xxxvii, 270.
(5) KEITH: Med. Press and Circ., 1915, xli, 72.
(6) ALVAREZ: This Journal, 1918, xlv, 346, 1918, xlvi, 555.
(7) CANNON: Ibid., 1902, vi, 265.
(8) ELLIOTT AND BARCLAY SMITH: Journ. Physiol., 1904, xxxi, 272.
(9) CASE: Proc. 17th Internat. Congr. Med., London, 1913, Sect. xxii, part ii, 14.
(10) ROST: Verhandl. d. deutsch. Gessellsch. f. Chir., 1912, 169.
SINGER AND HOLZKNECHT: Münch. med. Wochenschr., 1911, lviii, 2536.
KEITH: Brit. Journ. Surg., 1915, ii, 581.
(11) ALVAREZ: Journ. Amer. Med. Assoc., 1915, lxv, 393.

BLOOD VOLUME

A METHOD FOR ITS DETERMINATION WITH DATA FOR DOGS, CATS AND RABBITS

WALTER J. MEEK AND HERBERT S. GASSER

From the Physiological Laboratories of the University of Wisconsin and the Washington University Medical School

Received for publication October 9, 1918

Few problems in all physiology have been attacked more persistently or more ingeniously than that of determining the blood volume in mammals. The importance of such information from a theoretical as well as a practical standpoint has always been obvious. Although a score of methods have been described either for directly determining or indirectly calculating blood volume, we found that for work we wished to do on shock none completely met our needs. For example, very few of the methods allow a second determination to be made during the course of a single experiment. The final estimation of volume has usually depended on color comparisons, blood counts or other procedures in which certain errors if not absolutely necessary are, at least, easily made. It seemed to us that some method chemically accurate, gravimetric, if possible, might be of use if it could be devised.

Complete reviews of the blood volume literature may be found in the papers of Plesch (1), Behring (2), Dreyer and Ray (3) and Keith, Rowntree and Geraghty (4). It is here necessary only to comment on the various methods that have been employed.

The direct method of determining blood volume, that of bleeding and washing out the circulatory system, was introduced by Welcker (5) and used by many early investigators. With modifications it has been employed in recent years by Dreyer and his colleagues (3), (6), (7), Boycott (8), Rasmussen and Rasmussen (9) and others. Two possible errors have usually been recognized in the direct methods, namely: that pigment particularly from muscle might be included as hemoglobin and that some blood might not be washed out from the spleen, bone marrow or parts of the portal system. The final calculations must be made in all cases by comparing the washings with an original sample of blood in regard to hemoglobin, number of red blood cells or content of solids. Any error made in these comparisons of dilute solutions becomes magnified when the original volume is calculated. Although these criticisms may be made, the direct method as used by Dreyer and Ray is probably the most accurate means of determining blood volume yet devised. It is, of course, limited in application since the life of the animal is sacrificed.

Indirect methods have been more generally used. These all depend on the addition of some substance to the blood stream and a subsequent determination of the dilution or concentration undergone either by the blood or the substance itself. Thus Vierordt (10), who seems first to have used such a technique, bled an animal, waited until he believed the tissue juices had restored the lost fluid and then determined the dilution of the red blood cells. Assuming that the blood received from the tissues an amount exactly equal to the volume withdrawn before any erythrocytes were regenerated, the blood volume could, of course, be estimated. The method, however, rested on two assumptions and the exact facts regarding these are not even yet completely known. Much the same comments may be made on Vierordt's method depending on ventricular output and circulation time.

Valentin (11) computed volume from determinations of solids before and after an injection of a measured amount of water. His values were too high since either salts were drawn in from the tissues or a portion of the injected water left the blood stream. Malassez (12) injected serum or whole blood with a known red blood cell count into an animal and from the resulting variation in the number of red cells made his computations. This technique was ingenious but hemolysis often introduced a large error. Cohnstein and Zuntz (13), improving on Valentin's method, diluted the circulation with isotonic NaCl of a known quantity and from the red blood cell dilution determined the blood volume. Kottmann (14) followed much the same procedures but determined the red cells by means of the hematocrit. Oerum (15) also used the same general method but made his determinations from comparisons of the hemoglobin content of the diluted blood with that of the original. Changes in the specific gravity of the blood, brought about by injections of known amounts of NaCl, were used by Sherrington and Copeman (16) to determine blood volume. The chief difficulties of all these methods lie not only in the technical errors incident to blood counts, hematocrit and hemoglobin determinations but more especially in the extreme rapidity with which injected water and salt solutions leave the blood serum.

Two injection methods may be mentioned which differ from the preceding in that the substances injected do not leave the blood stream rapidly. Behring (2) investigated blood volume by studying the dilution undergone by a measured amount of tetanus antitoxin when injected into the blood. This method he recommended clinically.

A method of excellent promise has recently been described by Keith, Rowntree and Geraghty (4). It consists of an injection of a known amount of the dye, vital red, and a determination of its dilution in the blood. The dye is harmless in the doses employed and repeated determinations may be made. Although of great service clinically, in animal experiments the method often fails on account of slight hemolysis or lipaemia. In addition the dye cannot now be generally obtained.

Grehant and Quinaud (17) in 1883 introduced the CO inhalation method of determining blood volume. This consisted in breathing a known amount of CO and then determining the degree of saturation undergone by the hemoglobin of the blood. The CO capacity of the blood having previously been determined, the total volume could easily be calculated. Haldane and Smith (18) improved the method and later it was still further refined by Zuntz and Plesch (19) and Boycott and Douglas (20). The CO inhalation method has never given very constant results and, although it has been of some value clinically, it can scarcely be said to have a great degree of accuracy.

DETERMINATION OF BLOOD VOLUME WITH ACACIA

In casting about for some inert substance that could be injected into the circulation and then recovered, the conditions necessary for an indirect blood volume determination, the attention of one of us, Doctor Gasser, was directed to gum acacia. It seemed probable that this substance even in large doses might have no effect on any vital function and that it might be recovered from the blood as furfurol-phloroglucid by the method of Kröber (21). On experiment we found that both of these possibilities were true. Acacia may be injected into a dog intravenously until the animal's blood becomes a 7 per cent or 8 per cent solution without obvious changes in the respiratory or nervous systems, or in the circulatory, at least so far as can be told from general blood pressure. Under a local anaesthetic acacia may be injected into an intact animal until the blood is a 4 per cent solution without the general condition of the animal being in any way affected. After such injections acacia may be recovered as phloroglucid from a blood sample.

As a general plan for our method we proposed the following procedures: First, to determine the amount of acacia that could be recovered after adding a known amount to a 10 cc. sample of blood. This we shall speak of as the "control." Second, to inject such an amount of acacia into the animal that, on the assumption of his having a certain percentage of his body weight as blood, each 10 cc. of his blood would have exactly the same amount of acacia as the control. Third, allow ample time for the injected acacia to mix with the blood. Fourth, after time for mixing draw a 10 cc. sample of blood and determine its acacia content. This will be spoken of as the "blood sample." The assumed blood volume in percentage of body weight would then be to the true blood volume in percentage of body weight as the acacia content of the blood sample is to the acacia content of the control.

Acacia has in all our work been determined as furfurol-phloroglucid by the method of Kröber (21). We have made no important modification of his technique. It consists in distilling the acacia or blood containing acacia in the form of furfuraldehyde from HCl solution of specific gravity 1.06. To this distillate is added 1 per cent phloroglucin dissolved in HCl of specific gravity 1.06. The distillate should stand 24 hours to insure a complete precipitation of the phloroglucid. The precipitate is then caught on a Gooch crucible, dried four hours at 100°C., cooled in a desiccator and weighed. One hundred milligrams of acacia on the average yield about 26 mgm. of phloroglucid. After passing the crucible the filtrate is greenish in color showing that some of the phloroglucid is in solution. For 550 cc. of distillate Kröber estimates this amount to be 5.2 mgm. For the control and blood sample we must disregard this correction since, as shown later, the amount of phloroglucid recovered from these samples is in itself a constant fraction of the total amount of acacia added. To be sure that no more furfurol is coming over we have often let a drop of the distillate fall on a paper moistened with aniline acetate. A pink ring indicates the presence of furfuraldehyde. All determinations which had to do with establishing the method were done in duplicate. The method is in part empirical but it is accurate if the amount of phloroglucin added for precipitation is from 1.25 to 3 times the yield in phloroglucid. We have accordingly added from 5 to 10 cc. to our distillates.

In actual experiments we have found it most convenient to use acacia solutions of the following strengths. For our control we have added 10 cc. of 1 per cent acacia to 10 cc. of freshly drawn blood. The hydrochloric acid was not added until 10 minutes later. As an injection, we have generally given 4 cc. of a 20 per cent acacia solution for each kilo of body weight. The acacia for injection is made up in a 0.9 per cent NaCl solution. In some of our earlier experiments the acacia was dissolved in water. The presence or absence of the NaCl has seemed to make no difference in the determinations. If the animal should have 8.4 per cent of his body weight in blood then the amount injected would add exactly 10 mgm. of acacia to each cubic centimeter of his blood, an amount exactly equal to what has been added to the control. In all calculations the specific gravity of blood has been figured as 1.050. After the acacia determinations are made the blood volume may be calculated from the following proportions:

$$\frac{8.4 \text{ per cent}}{\text{Blood volume in per cent of body weight}} = \frac{\text{Weight of phloroglucid in blood sample}}{\text{Weight of phloroglucid in control}}$$

Or if the total volume is desired at once in cubic centimeters the proportion may be stated in this way:

$$\frac{20 \times \text{cc. of acacia injected}}{\text{Blood volume in cc.}} = \frac{\text{Weight of phloroglucid in blood sample}}{\text{Weight of phloroglucid in control}}$$

This volume must be corrected for the difference between the amount of blood drawn and the acacia injected.

In a number of experiments we determined the yield of the acacia itself in phloroglucid. From this we knew the total amount injected. Dividing this amount by the amount recovered in the blood sample gave us another means of determining blood volume. This formula was used to check up the other determinations.

Conditions which the method must satisfy. There are certain criticisms which the acacia method in common with all other indirect methods of determining blood pressure must meet. The most important of these are:

1. Can the acacia be recovered quantitatively from the blood?

2. Does any acacia leave the blood stream during the period allowed for mixing?

3. In the same period does the injected acacia in any way expand the blood volume?

In regard to the recovery of acacia from blood it was soon found that the total amount added could not be regained. To illustrate, if 10 cc. of 1 per cent was found to yield 26 mgm. of phloroglucid, the same amount in blood would produce only about 21 mgm. The amount lost varied with the kind of blood and with its quantity. More could be recovered from rabbits' blood than from dogs'. The percentage of acacia regained from varying amounts of blood may be seen from the following figures which are averages from a number of experiments.

10 cc. of 1 per cent acacia in 5 cc. blood yielded 90 per cent of total
10 cc. of 1 per cent acacia in 10 cc. blood yielded 83 per cent of total
10 cc. of 1 per cent acacia in 20 cc. blood yielded 76 per cent of total

We are unable to say with any certainty what becomes of this acacia. It is quite possible, however, that some of the aldehyde formed in the distillation may combine with substances in the blood and so fail to pass over. Pentoses are also known to form humin compounds with some of the amino acids.

At first sight it would seem that this loss of acacia, which is roughly proportional to the quantity of blood, would render our method impossible. To estimate the loss would require a knowledge of the blood volume, the very thing that the method is designed to discover. In actual practice, however, it is not the amount of blood which is the variable but the amount of acacia which a given quantity of blood receives on injection. If the blood volume should by chance be exactly equal to our assumed 8.4 per cent, then each 10 cc. of blood would receive an amount exactly equal to that in the control. Should the blood volume be either twice or one-half that expected, then each 10 cc. of blood would receive either one-half or twice that in the control. These figures represent the widest possible limits that might be expected for variations in blood volume. The important question would then seem to be whether on adding such amounts of acacia to 10 cc. samples of blood we can recover all or any constant fraction of the total. On taking 10 cc. quantities of blood and adding acacia to them in such amounts as the blood would receive in the body should it vary from twice to one-half normal, the following data were secured.

TABLE I

Showing the amount of acacia recovered as phloroglucid when 1 per cent acacia in varying quantities is added to 10 cc. samples of blood

EXPERIMENT NUMBER	5 CC. OF 1 PER CENT ACACIA IN 10 CC. BLOOD	10 CC. OF 1 PER CENT ACACIA IN 10 CC. BLOOD	20 CC. OF 1 PER CENT ACACIA IN 10 CC. BLOOD	30 CC. OF 1 PER CENT ACACIA IN 10 CC. BLOOD
	mgm.	*mgm.*	*mgm.*	*mgm.*
1	11.9	24.0	47.9	
2	12.2	24.6	50.9	
3	11.7	23.1	46.0	79.0
4	10.1		40.5	
5	12.3	25.3	54.6	
6	11.6	24.7	46.7	
7	11.8	23.2	50.6	78 0

These figures show clearly that almost exactly the same proportion of acacia may be recovered as phloroglucid from 10 cc. of blood no matter whether 5, 10 or 20 cc. of 1 per cent acacia have been added. Although 10 cc. of blood combine with more acacia when 20 cc. of the 1 per cent solution are added, the percentage yield remains the same for all three concentrations. The actual recovery in per cent may easily be determined in any of the experiments in which a preliminary analysis of the acacia alone was made. In experiment 1, 5 cc. of the 1 per cent acacia were analyzed and found to yield 15.8 mgm. of phloroglucid, including here, of course, the correction for the amount left in solution in the filtrate. The amounts recovered from 5, 10 and 20 cc. in blood were therefore 75.3 per cent, 75.9 per cent and 75.7 per cent. In experiment 2, 5 cc. of 1 per cent acacia alone yielded 15.55 mgm., the recovery for the three samples therefore being 78.4 per cent, 79.1 per cent and 81.8 per cent. All the experiments are not quite so satisfactory as these two but they all seem sufficiently well in agreement to justify our contention that the phloroglucid recovered from our control may be compared with that from the blood sample to determine volume even though the acacia in the two may vary in concentration. Most of the variations found in table 1 are really within the limits allowed for gravimetric methods. Variations of a few per cent here are fortunately almost without any influence on the final determinations of blood volume. In experiment 2, for example, there is a difference of 3.4 per cent between the percentage recovery of phloroglucid in the 5 and 20 cc. samples. It would take a difference of about 10 per cent, however, to make 0.5 per cent difference in the final determination of blood volume.

It cannot be denied that the conclusions just reached seem inconsistent with the evidence already submitted that with increasing amounts of blood we get decreasing yields of acacia as phloroglucid. This would seem to indicate that the blood contained some substance with which the acacia or one of its decomposition products united according to the general law of chemical proportions. If this were true on increasing the amount of acacia added to 10 cc. samples of blood we should expect to get a steady increase in the amount of acacia recovered as phloroglucid. On the contrary, we have found that within limits the same percentage of acacia is always recovered as phloroglucid from a given amount of blood. There is, however, a possible explanation for such a contradiction. As the concentration of the acacia in the 10 cc. samples increases it might form polymers with some unknown constituent of the blood instead of combining according to the law of chemical proportions. There are several facts that indicate such a possibility. Pentosans are known to polymerize in the formation of humin compounds. Furfurol has been shown to combine with phloroglucin in varying proportions according to the concentrations of each. There must, of course, some time be an end to the power of polymerizing and we should accordingly find a concentration of acacia that would give more than the expected yield. Twenty cubic centimeters of 1 per cent acacia in 10 cc. of blood is probably very near this limit. The yield for 20 cc. as shown by table 1 is, in most of the experiments, very close to four times that for 5 cc. but in several it is high and the average is somewhat above what is expected. When 30 cc. of 1 per cent acacia are added the limit in which we recover our expected amount is definitely passed and instead of the yield being 70.2 mgm. it is 79 mgm. Whatever the explanation may be the important thing for our method is the undoubted fact that we can regain exactly the same percentage of acacia as phloroglucid from a 10 cc. blood sample if it has anywhere from 5 to 20 cc. of 1 per cent acacia in it. These limits represent the concentrations found in blood volume determinations of one-half to two times normal.

The second condition that our method must satisfy is that no acacia shall be lost during the period allowed for mixing with the blood. It is of course, well known that many substances begin to leave the blood stream as soon as injected. Although this might not be expected of a compound having as large a molecule as acacia, it is a matter that must be determined as carefully as possible.

We have usually allowed ten minutes after injection for the acacia to mix thoroughly with the blood. This time has seemed longer than really necessary but we have preferred to be on the safe side. Keith, Rowntree and Geraghty (4) allowed three minutes for vital red to mix with the blood. Plesch (1) considered three to four minutes ample for mixing after injection of sodium chloride. Sherrington and Copeman (16) found mixing complete in the rabbit after as short a period as thirty to forty seconds. Acacia in a 20 per cent solution is a rather viscous fluid and could not be expected to mix as quickly as NaCl. Granting fifteen seconds as the circulation time, ten minutes would allow the acacia to travel through the body forty times, certainly opportunity for mingling completely with the blood. Our analyses not only justify such a conclusion but show that any time after five minutes the mixing is complete. The following table compares analyses of blood samples taken five and ten minutes after the injection of acacia was completed.

TABLE 2

Showing the amounts of acacia recovered as phloroglucid from samples taken at five and ten minute intervals after injection

EXPERIMENT NUMBER	FIVE MINUTE SAMPLE	TEN MINUTE SAMPLE
	mgm.	*mgm.*
1	20.0	20.6
2	20.9	20.6
3	23.5	24.1
4	20.8	20.0
5	17.5	17.5
6	20.4	18.6
7	17.6	17.6
8	22.5	22.9
9	19.4	19.7

With the exception of experiment 6, for which we have no explanation, these analyses show a very close agreement between the five and ten minute samples. Three important points are made clear by these data; first, that the acacia does mix thoroughly with the blood; second, that we have allowed ample time for mixing; and third, that during this period there is no loss of acacia from the blood stream.

Ultimately the acacia, of course, disappears from the blood. We have not attempted to work out the exact curve of its disappearance but a number of interesting observations have been made which may have some bearing on the use of acacia as an injection fluid. In one experiment after injection we found the following amounts present in 10 cc. samples at various times up to five hours.

	mgm.
3 minutes	15.9
12 minutes	15.8
18 minutes	15.1
60 minutes	14.4
5 hours	12.2

In this case the decrease at the end of the first hour was about 8.8 per cent and this increased to 22.8 per cent at the end of five hours. In another experiment at the end of twenty-four hours there was still present 50 per cent of the original amount injected. In two experiments in which perfectly normal dogs received intravenous injections of acacia in large doses, 12 to 15 cc. per kilo of a 20 per cent solution, there was found in the blood forty-eight hours later 42 per cent in one and 33 per cent in the other.

The third criticism which our method has to meet is that the acacia must not of itself expand the blood volume. This might be brought about mechanically by the injection of large quantities or by the osmotic properties of the acacia. The first of these is easily controlled. In large animals where the amount to be injected exceeds the blood drawn for the control we bleed an amount equal to the quantity to be injected. It is, of course, well known that after hemorrhages fluid is drawn in from the tissues to restore the depleted blood and that this process begins immediately. Sherrington and Copeman (16) found after drawing 32 cc. of blood from a six-pound rabbit that the maximum reduction in specific gravity was not reached until fifteen to twenty-five minutes afterward. From this it does not seem that drawing an amount of blood equal to the volume injected could introduce an appreciable error for our injection begins at once after the bleeding and it is finished usually within a few minutes. In small animals the amount drawn for controls may be greater than the amount of acacia to be injected. In these cases it is often convenient to inject 8 cc. per kilo of 10 per cent acacia instead of 4 cc. per kilo of 20 per cent.

That the osmotic pressure of the acacia has not influenced the blood volume during the mixing period is shown by the data in table 2. It is evident that there would be a constant decrease in the amounts recovered in the ten minute samples, if the blood volume were being expanded, unless the total expansion took place within the first five-minute period. Evidence obtained by following the hemoglobin shows, however, that this is not the case. This conclusion is also borne out by numerous blood counts. At the end of the mixing period there has never been a decrease in the red blood count that was not almost entirely accounted for by the direct dilution resulting from the injection. In some cases there has been a slight increase after injection, the meaning of which is not clear. In table 3 are given the data covering these points.

TABLE

RED BLOOD CELL COUNT BEFORE INJECTION	ESTIMATED DILUTION OF THE BLOOD DUE TO THE VOLUME OF ACACIA INJECTED	RED BLOOD CELL COUNT 10 MINUTES AFTER INJECTION.	DECREASE IN RED BLOOD CELLS.
	per cent		*per cent*
8,320,000	10	7,520,000	9
8,640,000	16	8,800,000	Increase
4,650,000	5	4,792,000	Increase
4,368,000	9	4,032,000	7
5,800,000	12	5,008,000	13
7,040,000	15	6,236,000	11
8,440,000	9	7,600,000	9
6,583,000	13	5,405,000	17
6,700,000	19	5,840,000	12
8,750,000	4	8,500,000	3

Even after the mixing period no further dilution appears in the blood at least for a considerable period of time. Data from two experiments covering this point are here given.

December 2. Dog

10.10 a.m.	Blood count equals	6,016,000
10.34 a.m.	Acacia injected	
10.40 a.m.	Blood count equals	5,608,000
10.59 a.m.	Blood count equals	5,920,000
11.40 a.m.	Blood count equals	5,536,000

December 12. Dog

9.45 a.m.	Blood count equals	6,600,000
10.00 a.m.	Acacia injected	
10.37 a.m.	Blood count equals	6,400,000

A 20 per cent solution of acacia in a 0.9 per cent NaCl has, however, an osmotic pressure appreciably higher than that of the blood serum and it would seem that sooner or later this influence would exert itself by drawing in water from the tissues. In certain experiments we have evidence that in the course of an hour or two this really may take place. Our injection of 4 cc. per kilo of 20 per cent acacia converts the plasma of a dog into a 1.38 per cent acacia solution on the basis of the plasma being 60 per cent of the blood and the total blood volume being about 9 per cent of the body weight. Two things would, of course, happen to the plasma in this case. Its osmotic pressure would be lowered by the dilution undergone on adding the acacia, but this would be more than compensated for by the increase due to the higher osmotic pressure of the acacia. The dilution would be about 1/13.5 (7.4 per cent) of the plasma volume. This would lower the colloidal osmotic pressure of the plasma about 2.70 mm. Hg. using Bayliss' (22) figures for the osmotic pressure of serum. Bayliss found 7 per cent sodium acacia to have an osmotic pressure of 39 mm. Hg. If calcium acacia is the same, a 1.38 per cent solution would have a pressure of 7.7 mm. Hg. The plasma would thus have its colloidal pressure lowered 2.70 mm. and raised 7.7 mm. bringing it to 45 mm. Hg. This is an increase of 12.5 per cent above the original. We might then expect a plasma dilution of about the same percentage due to the inflow of tissue juices. But the plasma is only about 60 per cent of the total blood volume; therefore the total blood volume could be expanded only about 7.5 per cent. It should be recalled at this point that we have already submitted data showing that at the end of an hour something like 9 to 10 per cent of the acacia had left the blood stream. Data for only one experiment have been given but the results are in harmony with those of several others. This decrease in acacia is due only in part to expansion of the blood, since the maximum dilution as determined by the hemoglobin content of the blood has never been found to be greater than 7.5 per cent, an amount which corresponds closely to the calculated theoretical expansion. Data covering this point may be seen in the following table.

It will be noticed from table 4 that the maximum expansion occurs before the end of an hour. The 9 to 10 per cent decrease in the acacia of the blood stream is, therefore, in part a real loss and additional evidence of this fact is afforded by a reaction for pentose which may be obtained from the urine at this time. From the foregoing data and discussion it is evident that the osmotic properties of the acacia are not a seriously disturbing element in the determination of blood volume.

Second determinations of blood volume in the same animal. To be most useful a blood volume method should be capable of being used at frequent intervals on the same animal. The acacia method may be

TABLE 4

Increase in volume of blood as determined by the hemoglobin content at various periods after injection

EXPERIMENT NUMBER	MINUTES AFTER INJECTION	INCREASE IN BLOOD VOLUME
		per cent
47	10	5.6
	30	6.6
	60	6.0
48	30	3.1
49	39	−2.8
50	6	7.3
	25	7.5
51	27	4.0

used at least twice successfully in the same experiment. For the second control 10 cc. of blood are drawn and to it are added 5 cc. of the 1 per cent acacia solution. An injection equal to one-half the first is now made, the usual time for mixing allowed and then the second blood sample drawn. The acacia content of these samples as phloroglucid is then determined. In order to make use of these data the amount of acacia remaining in the blood from the first injection must be known. This is easily found by subtracting one-half of the first control from the second. If this difference is now subtracted from the second blood sample, the equation may be completed as follows:

$$\frac{8.4 \text{ per cent}}{\text{Blood volume in per cent of body weight}} = \frac{\text{Pg. in second blood sample} - (\text{Pg. in second control} - \tfrac{1}{2}\text{ first control})}{\tfrac{1}{2}\text{ First control}}$$

Immediately succeeding determinations will be found to correspond if the analyses are done in duplicate and the duplicates check each other closely. The necessity of perfection in technique is apparent when one considers that an error of only 0.1 mgm. may change the second blood volume as much as 0.1 per cent of body weight. This is due to the small weights compared in the ratio used for the second determination. In table 5 are given the results of three such determinations.

In tables 6, 7, 8, may be found the data from a number of blood volume determinations on dogs, cats and rabbits. We have made no particular effort to compare these results with those of previous workers. Plesch found the average blood volume in nine healthy dogs was

TABLE 5

EXPERIMENT	FIRST CONTROL	FIRST BLOOD SAMPLE	SECOND CONTROL	SECOND BLOOD	FIRST BLOOD VOLUME	SECOND BLOOD VOLUME
	mgm.	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
20	23.3	23.5	33.3	33.3	8.7	8.8
21	24.1	23.2	35.7	34.5	9.1	9.7
22	23.9	20.9	33.0	32.0	10.0	9.6

TABLE 6

Blood volume of dogs in percentage of body weight.

WEIGHT IN	PHLORO-GLUCID IN CONTROL	PHLORO-GLUCID IN BLOOD SAMPLE	CORRECTION FOR BLEEDING AND INJECTING	BLOOD VOLUME IN PER CENT OF BODY WEIGHT
kgm.	*mgm.*	*mgm.*	*cc.*	*per cent*
8.80	24.1	21.2	−15.2	9.36
8.00	25.0	19.5	− 8.0	10.65
7.56	23.9	22.6	− 5.2	8.80
4.60	20.0	18.8	−12.8	8.60
9.51	18.5	15.6	−13.0	9.81
5.10	19.4	19.7	+ 1.6	8.28
8.40	22.6	20.8	0.0*	9.56
5.02	21.2	15.7	+ 2.0	11.38
4.84	27.6	21.1	+ 5.7	11.02
5.40	21.1	18.4	− 1.6	9.59
7.80	23.3	23.5	0.0*	8.72
5.42	22.6	23.1	+53.4	9.24
8.28	23.8	20.4	− 7.1	9.69
12.06	28.8	23.1	−28.3	10.15
11.20	20.1	15.8	−29.8	10.32
13.20	23.1	18.6	0.0	10.43
9.40	20.9	17.2	0.0	10.20
20.00	24.0	22.3	0.0*	9.47
Average				9.74

*In these experiments 12 cc. per kilo of 7 per cent acacia were used; 8.4 of the formula was therefore replaced by 8.8.

8.57 per cent of body weight with variations from 7.42 per cent to 10.46 per cent. For rabbits, Dreyer and Ray give the blood volume as 4.92 per cent of body weight although it should be noted that they do not believe the ratio of blood volume to body weight is a constant in normal animals of any given species. Our results are therefore somewhat higher than any previously reported.

TABLE 7

Blood volume of cats in percentage of body weight

WEIGHT IN	PHLORO-GLUCID IN CONTROL	PHLORO-GLUCID IN BLOOD SAMPLE	CORRECTION FOR BLEEDING AND INJECTING	BLOOD VOLUME IN PER CENT OF BODY WEIGHT
kgm.	*mgm.*	*mgm.*	*cc.*	*per cent*
3.44	22.2	37.8	+13.3	5.33
2.75	20.7	27.9	+14.0	6.76
3.90	20.9	31.7	+ 7.4	5.72
3.50	23.6	41.0	+ 6.0	5.00
2.72	21.0	35.0	0.0	5.04
1.50	21.6	30.8	+ 5.0	6.23
2.20	23.1	42.8	0.0	4.53
4.30	16.0	26.0	0.0	5.41
Average				5.50

Note: 7 per cent acacia was used in the last experiment of the table.

TABLE 8

Blood volume of rabbits in percentage of body weight

WEIGHT IN	PHLORO-GLUCID IN CONTROL	PHLORO-GLUCID IN BLOOD SAMPLE	CORRECTION FOR BLEEDING AND INJECTING	BLOOD VOLUME IN PER CENT OF BODY WEIGHT
kgm.	*mgm.*	*mgm.*	*cc.*	*per cent*
2.00	21.2	36.5	+6.0	5.18
3.00	18.3	34.9	−2.0	4.32
2.50	24.6	34.3	+2.0	6.10
1.80	23.8	42.8	0.0	4.67
2.10	27.0	38.8	0.0	5.84
2.57	30.2	48.1	+6.4	5.42
2.17	34.2	45.0	+3.6	6.56
Average				5.44

SUMMARY

A method of blood volume determination by means of an acacia injection has been reported. The method depends on an injection of a known amount of acacia into the blood stream and a determination of the dilution undergone by the acacia after time for mixing with the blood has been allowed. The acacia has been determined as furfurol-phloroglucid by the method of Körber. The injection of 4 cc. of 20 per cent acacia per kilo has been shown to be harmless to the animal, neither does it expand the blood volume during the time necessary for a determination.

By this method the average blood volume of the dog has been found to average 9.72 per cent of the body weight. For the cat and rabbit the percentages are 5.50 and 5.44 respectively.

The authors wish to thank two of their students, Mr. A. E. Cole and Mr. A. R. Langjahr, for help in making analyses and performing experiments.

BIBLIOGRAPHY

(1) PLESCH: Zeitsch. f. exper. Path. u. Therap., 1907, vi, 380.
(2) BEHRING: Beitr. z. exper. Therap., Heft 12, 1912.
(3) DREYER AND RAY: Phil. Trans. Roy. Soc., 1910, cci, 133.
(4) KEITH, ROWNTREE AND GERAGHTY: Arch. Int. Med., 1915, xvi, 547.
(5) WELCKER: Prag. Vierteljahrschr., 1854, iv, 145.
(6) DREYER, RAY AND WALKER: Skand. Arch. f. Physiol., 1913, xxviii, 299.
(7) DREYER, RAY AND WALKER: Journ. Path. and Bact., 1911, xvii, 143.
(8) BOYCOTT: Journ. Path. and Bact., 1911, xvi, 485.
(9) RASMUSSEN AND RASMUSSEN: This Journal, 1917, xliv, 132.
(10) VIERORDT: Arch. f. physiol. Heilk. N. F., 1858, ii, 527.
(11) VALENTIN: Versuch einer physiologisches Pathologie des Herz, Leipsig, 1866.
(12) MALASSEZ: Arch. d. Physiol., 1874, i, 797.
(13) COHNSTEIN UND ZUNTZ: Pflüger's Arch., 1884, xxxiv, 191; 1888, xlii, 303.
(14) KOTTMAN: Arch. exper. Path. u. Pharm., 1908, liv, 357.
(15) OERUM: Quoted from Plesch.
(16) SHERRINGTON AND COPEMAN: Journ. Physiol., 1893, xiv, 52.
(17) GREHANT AND QUINAUD: Journ. Anat. et Physiol., 1883, xviii, 564.
(18) HALDANE AND SMITH: Journ. Physiol., xxv, 331.
(19) ZUNTZ AND PLESCH: Biochem. Zeitschr., 1908, xi, 47.
(20) BOYCOTT AND DOUGLAS: Journ. Path. and Bact., xiii, 256.
(21) KRÖBER: Journ. f. Landwirtschaft., 1900, xlviii, 357.
(22) BAYLISS: Proc. Roy. Soc., 1916, lxxxix, 380.

QUANTITATIVE STUDIES ON INTRACELLULAR RESPIRATION

IV. The Rates of CO_2 Production by Starved and Fed Paramecia, and their Possible Relation to the Rates of Oxidation in the Unfertilized and Fertilized Sea Urchins Egg

E. J. LUND

From the Department of Animal Biology, University of Minnesota

Received for publication October 17, 1918

The purpose of the present paper is *a*, to present data which show that the carbon dioxide production by Paramecium is increased very markedly after ingestion of food such as yeast or egg yolk, thus confirming the results presented in the previous paper on the relation of state of nutrition and rate of oxygen consumption; and *b*, to show that this marked increase in respiration is not necessarily accompanied by cell division, thus being closely parallel to the condition found by others in the egg of the sea urchin.

For determining such very small quantities of carbon dioxide as are produced by 1 or 2 cc. of a suspension of Paramecium, a method was employed, the description of which has been given elsewhere together with tests of its accuracy (1).

The conclusions from results of the experiments reported below, using this method, do not depend for their validity upon the question as to whether the method is capable of measuring the absolute quantity of carbon dioxide produced by the organisms, for the measurements of the carbon dioxide produced by the fed and starved Paramecia are primarily comparative, which is all that is required for establishing the conclusions. As a matter of fact, the results very probably also represent the absolute quantities of CO_2 produced by the Paramecia during the periods of the experiments, within limits of error of the method when such small amounts of carbon dioxide are measured. When the method is properly carried out, the error in measuring amounts of carbon dioxide equal to that liberated from 1 mgm. of Na_2CO_3 by an acid is about equal to 5 per cent.

The results of four experiments are given in the following four tables. The procedure was practically the same in all of them. They differed only in the degree of starvation before the experiments were begun and in the number of Paramecia tested in each experiment. The description of the plan and procedure of the experiment, table 1, will apply to all the others except for minor differences which are noted in the description above each of the tables. Paramecia from a pure line were transferred gradually from native hay infusion to tap water and starved for various periods of time. This suspension was concentrated with the centrifuge just before the beginning of the experiment. The words "native tap" in columns 3 and 5 of the tables mean the tap water medium into which the Paramecia had finally been transferred after washing. Fresh compressed yeast suspended in fifty times its

TABLE 1

Paramecia were starved 24 hours before being used. The protoplasm was clear. Figures refer to the number of cubic centimeters n/100.3 HCl *necessary for neutralization of the free* Ba(OH)$_2$ *in each bottle. Twenty cubic centimeters* Ba(OH)$_2$ *were added to each bottle. Duration of experiment, 23 hours. Temperature 26°C.*

BOTTLE	TITRATED AT ONCE	TITRATED AT END OF 23 HOURS			
	Controls. No yeast added			Yeast added	
	1	2	3	4	5
	Blanks	2 cc. Paramecia + 3 drops tap water. Starved	2 cc. "native tap" + 3 drops tap water. No Paramecia	2 cc. Paramecia + 3 drops yeast. Fed	2 cc. "native tap" + 3 drops yeast. No Paramecia
1	21.40	19.60	20.15	18.80	20.15
2	21.35	19.55	20.30	18.80	20.20
3	21.40	19.57			20.12
Average.	21.38	19.57	20.22	18.80	20.15

	cc.
Average cc. n/100.3 HCl equivalent of CO_2 absorbed from air in bottle and 2 cc. "native tap." Difference between columns 1 and 3	1.16
Average cc. n/100.3 HCl equivalent of CO_2 produced by dead yeast. Difference between averages, columns 3 and 5	0.07
Average cc. n/100.3 HCl equivalent of CO_2 produced by starved Paramecia. Difference between averages, columns 2 and 3	0.65
Average cc. n/100.3 HCl equivalent of CO_2 produced by fed Paramecia. Difference between averages, columns 4 and 5	1.35

volume of distilled water was killed by boiling for one minute, quickly cooled and then washed in tap water by means of the centrifuge. This suspension of yeast was used as food for the animals. The figures in table 1, column 1, are the number of cubic centimeters n/100.3 HCl which were necessary for neutralization of 20 cc. of the stock solution of $Ba(OH)_2$, i.e., before absorption of any CO_2. The procedure in adding the $Ba(OH)_2$ solution to the bottles and titration has been given in the description of the method.

The numbers in columns 2, 3, 4 and 5 give the number of cubic centimeters n/100.3 HCl necessary to neutralize the remaining free base after absorption of all CO_2 liberated from various sources, as indicated in the separate columns, during the period of the experiment which was in this case twenty-three hours. The average CO_2 produced by the unfed Paramecia in 2 cc. of the concentrated Paramecium suspension in tap water during twenty-three hours is equal to the difference between the averages in columns 2 and 3. The average in column 3 is a control to determine how much free $Ba(OH)_2$ in terms of cubic centimeters n/100.3 HCl remained after all free CO_2 from the air in the bottles and the dissolved CO_2 in the 2 cc. tap water medium in which the Paramecia lived (designated "native tap" in the tables), had been absorbed.

The bottles in column 2 were set up exactly like those in column 3 except that Paramecia were present. It is therefore obvious that the numbers in column 2 represent the amount of free $Ba(OH)_2$ in terms of cubic centimeters n/100.3 HCl which remained after all the free CO_2 present in the air in the bottle, plus that amount which was dissolved in the 2 cc. tap water medium introduced with the Paramecia, *plus the amount of CO_2 produced by the Paramecia in 2 cc. of the stock suspension, during 23 hours,* had been absorbed. The figures in column 2 are smaller than those in column 3, thus showing that more $Ba(OH)_2$ had been neutralized in these bottles than in those of column 3, i.e., more CO_2 had been absorbed in bottles of column 2 than in those in column 3. This difference in cubic centimeters n/100.3 HCl is therefore equivalent to the amount of CO_2 produced by the unfed Paramecia in 2 cc. of the Paramecium suspension during 23 hours. In this experiment the CO_2 produced by the starved animals was equivalent to 0.65 cc. n/100.3 HCl.

The bottles in columns 4 and 5 are *exact duplicates* respectively of the bottles in columns 2 and 3, except that food (three drops of the suspension of yeast cells in tap water) was added. The figures in column 5 therefore give the amount of free $Ba(OH)_2$ remaining after all CO_2 present in the air in the bottles, plus the CO_2 dissolved in the 2 cc. tap water medium, plus any CO_2 produced by the killed yeast cells, had been absorbed. That the killed yeast did not produce any CO_2 is shown by the fact that the average amount of $Ba(OH)_2$ left in the bottles in columns 3 and 5 is the same within the limits of error in titration. The average amount of CO_2 produced by the fed Paramecia, column 4, is therefore equivalent to the difference between 20.15 cc. n/100.3 HCl, column 5, and 18.80 cc. n/100.3 HCl, column 4, or 1.35 cc. n/100.3 HCl. The quantity of CO_2 produced by the starved Paramecia was therefore only one-half as much as that produced by the same number of fed Paramecia during the same period, all other conditions being identical.

Both the actual amounts of CO_2 produced by the Paramecia in terms of cubic centimeters n/100.3 HCl—0.65 cc. and 1.35 cc. respectively—and the difference in these same amounts, or 0.70 cc., are several times greater than any errors entering into the titrations or other sources of error in any part of the experiment. The maximum difference between any two of the duplicate titrations is 0.15 cc. n/100.3 HCl. Taking averages of the duplicate tests reduces this source of error to one-half or one-third. This degree of uniformity in the results completely justifies confidence in the method employed and confirms its reliability for determining such small amounts of CO_2 as are dealt with in these experiments on Paramecium and as was shown in tests of the method by using known amounts of CO_2 in the form of Na_2CO_3 (1).

Furthermore, the following tables show an equal degree of accuracy in the results. The number of Paramecia in each 2 cc. of suspension must be large enough so as to produce a sufficient amount of CO_2 for measurement. The smallest quantity of CO_2 which can be measured to within about 5 per cent error, is roughly equal to the amount of CO_2 set free from 1 mgm. of Na_2CO_3 by an acid, while for merely detection of differences this amount of CO_2 may be as small as the amount of CO_2 equivalent to 0.1 to 0.2 cc. n/100 HCl, or the amount of CO_2 set free from about 0.05 to 0.1 mgm. of Na_2CO_3 by an acid. This assumes of course that the technique of the experimenter in carrying out the experiment is satisfactory and as described. Fortunately in this method the accuracy of procedure is indicated by the degree of uniformity in the titrations of the free $Ba(OH)_2$ in the various duplicate bottles.

The experiment in table 2 is practically a repetition of the one in table 1 except that a different suspension of Paramecia was used; the duration of the experiment was 22 instead of 23 hours and the temperature was 24°C. instead of 26°C. The average amounts of CO_2 produced by equal numbers of starved and fed Paramecia were respectively equivalent to 0.44 cc. and 0.83 cc. n/100.3 HCl, hence the amount of CO_2 produced by the Paramecia fed with yeast was about twice as great as that produced by the same number of starved ones in the same length of time.

TABLE 2

Paramecia starved 48 hours in tap water previous to the experiment. Protoplasm was transparent. Twenty cubic centimeters $Ba(OH)_2$ *were added to each bottle. Duration of experiment, 22 hours. Figures in the table represent the number of cubic centimeters n/*100.3 HCl *required to neutralize the free* $Ba(OH)_2$ *in each bottle. Temperature 24°C.*

BOTTLE	TITRATED AT ONCE	TITRATED AT END OF 22 HOURS			
		Controls. No yeast added		Yeast added	
	1	2	3	4	5
	Blanks	2 cc. Paramecia + 3 drops tap water. Starved	2 cc. "native tap" + 3 drops tap water. No Paramecium	2 cc. Paramecia + 3 drops yeast. Fed	2 cc. "native tap" + 3 drops yeast. No Paramecium. Control
1	21.00	19.60	20.05	19.15	19.90
2	20.95	19.50	19.98	19.12	19.95
3	20.90	19.60		19.05	19.95
Average	20.95	19.57	20.01	19.11	19.94

	cc.
Average cc. n/100.3 HCl equivalent of CO_2 absorbed from air in the bottle and 2 cc. "native tap." Difference between columns 1 and 3	0.94
Average cc. n/100.3 HCl equivalent of CO_2 produced by dead yeast. Difference between averages in columns 3 and 5	0.07
Average cc. n/100.3 HCl equivalent of CO_2 produced by starved Paramecium. Difference between averages in columns 2 and 3	0.44
Average cc. n/100.3 HCl equivalent of CO_2 produced by fed Paramecium. Difference between averages in columns 4 and 5	0.83
Greatest difference in any duplicate titration	0.10

In order to determine whether the increased CO_2 production by fed Paramecia was specifically associated with and a result of cell division as such, or whether it was a result of ingestion, digestion and assimilation of the food by the cell leading to growth but *not cell divi-*

sion as such, the duration of the experiment was shortened to 8 and 9 hours so as to determine the CO_2 produced by the starved and fed Paramecia *before* any divisions of cells had taken place.

That cell division does not take place under the conditions of these experiments was shown in the previous paper on the relation of oxygen consumption to state of nutrition. In addition to the reasons given in the previous paper for believing that no cell division took place within eight to nine hours under the conditions of the experiments, it *was shown by direct counts of the number of Paramecia that the fed ones had not increased in number during this period.* From the results of the experiment in table 4 (really a double experiment, using yeast and yolk of hen's egg as food in two parts of the experiment) it is seen that the amount of CO_2 produced by the yeast-fed Paramecia was equivalent to 1.19 cc. n/100.3 HCl, while that of the starved ones was equivalent to 0.35 cc. n/100.3 HCl, or over three times as much CO_2 was produced by the yeast-fed Paramecia in 8 hours as by the same number of starved ones. Those fed with yolk produced two and one-half times as much CO_2 as the starved ones. In other words, both yolk and boiled yeast give the same result. Counts showed that no increase in the number of Paramecia during the 8 hours had occurred, hence it is clearly proved that cell division as such is not responsible for the observed increase of CO_2 production with ingestion of food by the cell.

The results of the experiment in table 3 are similar but owing to the small number of Paramecia in each 4 cc. of the Paramecium suspension used, the quantities of CO_2 produced by the Paramecia are too small for drawing definite conclusions. The table is added to show the degree of uniformity obtainable and to indicate the minimal quantities of CO_2 which can be dealt with by the method.

The above experiments on CO_2 production confirm in every respect those on the consumption of oxygen by fed and starving Paramecia when Winkler's method for oxygen determination was used, and will serve as a secure basis for interpretation of the relations of oxidations to other cell processes in Paramecium (2). It is interesting to note that the magnitude of the increase in rate of oxidations after feeding a starved Paramecium is similar, although somewhat greater, than that found in animals such as the cat, dog or man after feeding protein (3).

Significance of acceleration of oxidations in the fertilized echinoderm egg, in the light of these facts. It is a well-known fact that the rate of oxidations in the egg of the sea urchin is increased several fold as a result of fertilization, and Warburg (4) has further shown that this increase in rate of oxidation need not be followed by cell division for when eggs were placed in dilute solutions of phenylurethane after fertilization, cell division was inhibited while the increase in the rate of oxidations was not prevented. However, in some of the fertilized eggs treated with phenylurethane, protoplasmic movements and nuclear changes occur to a greater or less extent, and under certain conditions monasters are formed and even attempts at cell division (5). Hence for the sea urchin's egg the question is not quite clear as to whether increase in the rate of oxidation is entirely independent of the mitotic process in one form or other. In Paramecium it is certain

TABLE 3

Paramecium starved 48 hours in tap water. Duration only 9 hours in order to eliminate any possible effects of cell division in fed lots. The total number of Paramecia were too few to give any marked difference in CO_2 *production between fed and starved animals in spite of the fact that 4 cc. of the Paramecium suspension were used instead of 2 cc. as in the previous experiments. Other conditions same as in tables 1 and 2.*

BOTTLE	TITRATED AT ONCE	TITRATED AT END OF 9 HOURS			
		Controls. No yeast added		Yeast added	
	1	2	3	4	5
	Blanks	4 cc. Paramecia + 3 drops tap water. Starved	4 cc. "native tap" + 3 drops tap water. No Paramecium	4 cc. Paramecia + 3 drops yeast Fed	4 cc "native tap" + 3 drops yeast. No Paramecia. Control
1	21.05	20.25	20.25	20.08	20.31
2	21.05	20.25	20.30	20.12	20.26
3			20.32	20.15	20.33
Average.	21.05	20.25	20.29	20.11	20.30

	cc.
Average number cc. n, 100.3 HCl equivalent of CO_2 absorbed from air in bottle and 4 cc. "native tap." Difference between columns 1 and 3	0.76
Average number cc. n 100.3 HCl equivalent of CO_2 produced by dead yeast. Difference between columns 3 and 5	0.01
Average number cc. n 100.3 HCl equivalent of CO_2 produced by starved Paramecium. Difference between columns 2 and 3	0.04
Average number cc. n 100.3 HCl equivalent of CO_2 produced by fed Paramecium. Difference between columns 4 and 5	0.19
Greatest difference in duplicate titrations	0.07

that cell division does not necessarily accompany the marked rise in rate of oxidations after feeding. If the same degree of independence of acceleration of oxidations and cell division occurs in the sea urchin egg as in Paramecium, then the means by which the sperm or other agent increases the oxidations in the egg may be by *making available*

TABLE 4

Paramecia transferred slowly to pure tap water and left to starve for 15 hours. Then concentrated to 30 cc. Four cubic centimeters of this suspension were used for each test. Counts showed that in each 4 cc. there were from 28,000 to 30,000 Paramecia or from fifteen to twenty times the number present in each 4 cc. of the suspension used in experiment, table 3. Fifteen cubic centimeters $Ba(OH)_2$ *were added to each bottle, otherwise procedure was the same as in experiment, table 3. A test using yolk of hen's egg as food instead of yeast is given in columns 6 and 7. The yolk suspension was made by diluting fresh yolk with twenty-five volumes of distilled water.*

BOTTLE	TITRATED AT ONCE	TITRATED AT END OF 8 HOURS					
	Controls. No yeast added			Yeast added		Fresh yolk of hen's egg added	
	1	2	3	4	5	6	7
	Blanks	4 cc. Paramecia + 3 drops tap water. Starved	4 cc. "native tap" + 3 drops tap water. No Paramecium	4 cc. Paramecia + 3 drops yeast. Fed.	4 cc. "native tap" + 3 drops yeast. No Paramecia. Control.	4 cc. Paramecia + 4 drops yolk. Fed.	4 cc. "native tap" + 4 drops yolk. No Paramecia
1	15.85	14.71	15.00	13.85	15.15	14.00	15.00
2	15.72	14.63	15.00	13.80	14.95	14.05	15.05
3	15.71	14.70	15.08	13.91	15.02	14.20	15.02
Average..	15.76	14.68	15.03	13.85	15.04	14.08	15.02

	cc.
Average number of cc. n/100.3 HCl equivalent of CO_2 absorbed from air in bottle and 4 cc. "native tap." Difference between columns 1 and 3	0.73
Average number cc. n/100.3 HCl equivalent of CO_2 produced by dead yeast. Difference between columns 3 and 5.	0.01
Average number of cc. n/100.3 HCl equivalent of CO_2 produced by starved Paramecia. Difference between columns 2 and 3	0.35
Average number of cc. n/100.3 HCl equivalent of CO_2 produced by Paramecia fed with yeast. Difference between columns 4 and 5	1.19
Average number of cc. n/100.3 HCl equivalent of CO_2 produced by Paramecia fed with yolk. Difference between columns 6 and 7	0.94
Greatest difference in duplicate titrations	0.20

for assimilation and growth the deutoplasmic food (yolk) reserve of the cell, just as ingestion of food into the food vacuole of Paramecium makes possible assimilation and growth and hence doubling or trebling of rate of oxidations. That the causes for an increase in rate of oxidations in the sea urchin's egg after fertilization and in Paramecium after feeding are the same seems furthermore entirely probable, for the following reasons. It has been definitely shown that increase in permeability of the surface protoplasm of the egg follows fertilization, and while we have no such clear evidence as to changes in permeability in the *internal* plasma membranes of the egg (phase boundaries between different components of protoplasm, such as alveolar membranes) at the time of cleavage, yet these phase boundaries of the internal parts of the cell undoubtedly undergo similar changes in permeability. One of the fundamental properties of protoplasm is its development of internal surface. If we are able to apply, as seems very probable, the facts and conceptions of permeability derived from study of the outer cell surface to internal cell membranes, such as walls of the alveoli which are so common a feature of cytoplasmic structure, then we have gone a long way toward discovering a mechanism which will account in a rational way for many well-known but isolated facts. One of these is the differential distribution of substances in the cell, such as pigments and different solutes in different vacuoles of the same cell. The internal plasma membranes and phase boundaries evidently serve as the means of separating and preventing an intermixing of these solutes. Internal plasma membranes because of their peculiar permeability properties make possible cellular *differentiation of the cell solution*. If admixture of these substances (e.g., enzymes and their substrates) are made possible by an increase in permeability of these internal plasma membranes, then chemical changes are initiated. In this way it is easy to conceive of the internal, differentially semipermeable plasma membrane system as being the main controlling mechanism for at least certain of the chemical and physical processes in the cell. From this viewpoint it is readily seen that the yolk or food reserve of the egg (echinoderm) may not differ in its relation to the metabolism of the egg in any essential respects from the food reserve and ingested food of Paramecium. Fertilization in the egg involves increase in permeability of the limiting plasma membranes around the yolk particles in the cytoplasm, which leads to digestion, absorption and synthesis of living protoplasm and hence increase in rate of oxidations just as occurs in Paramecium. In Paramecium and still better known for Bursaria (a ciliate similar to Paramecium) it has been definitely shown that the permeability of different food vacuole membranes differs and further that the same plasma membrane differs in its degree of permeability at different times during the process of digestion and absorption of the food contents. Furthermore this permeability is in this case to some extent selective (6). If the plasma membranes of the food vacuoles of Bursaria possess these properties is it not very probable that similar capacities for change should be present in the internal membranes of the sea urchin egg and other cells?

The results from the study of Paramecium in this and the previous paper, together with the above interpretation of the possible rôle of internal plasma membranes in the echinoderm egg at the time of fertilization, place the phenomenon of the marked increase in oxidation in Paramecium after feeding and the similar increase in rate of oxidation in the sea urchin egg after fertilization on the same basis and subject to the same interpretation, thus making clear the significance of the several-fold increase in rate of oxidations of the sea urchin's egg after fertilization.

SUMMARY

1. Feeding a starving Paramecium with yeast or yolk of hen's egg increases the rate of CO_2 production from two to three times, thus confirming the previous results on the measurements of the rates of oxygen consumption.

2. This acceleration of the oxidations occurs in the absence of cell division. The process of cell division as such in all probability is not associated with any marked change in the rate of oxidations.

3. The immediate conditions which underlie the difference in rates of oxidation in the unfertilized and fertilized sea urchin's egg are in all probability the same as those underlying the difference in rate of oxidations in a starving and well-nourished Paramecium.

4. The results from experiments on Paramecium show that the method can be used for determining very small quantities of carbon dioxide produced by unicellular or other lower organisms.

BIBLIOGRAPHY

(1) Lund: Biol. Bull., 1918, xxxv.
(2) Lund: Biol. Bull., 1918, xxxv, 211.
(3) Lusk: Science of nutrition, 3d ed., 1917.
(4) Warburg: Zeitschr. f. Physiol. Chem., 1911, lxx.
(5) Painter: Journ. Exper. Zoöl., 1918, xxiv, 445.
(6) Lund: Journ. Exper. Zoöl., 1914, xvii, 1.

TWO NEW FACTORS IN BLOOD COAGULATION—HEPARIN AND PRO-ANTITHROMBIN

W. H. HOWELL AND EMMETT HOLT

From the Physiological Laboratory of the Johns Hopkins University

Received for publication October 17, 1918

A survey of the results of recent work indicates that at least six different substances are concerned in one way or another in the process of blood coagulation; namely, fibrinogen, thrombin, prothrombin, calcium, antithrombin and the so-called zymoplastic or thromboplastic substances furnished by the body cells in general, including the blood corpuscles. With regard to the last mentioned factor satisfactory evidence has been obtained to show that the active material in tissue extracts is a phosphatid (1) (cephalin). Work that has been going on in this laboratory during the past two or three years shows that we must add two other substances to this list of fibrin factors. So far as we know, neither of these substances has been recognized by other workers.

The two new factors are: First, a phosphatid, not previously described, which exists in various tissues but is found in greatest abundance in the liver. This phosphatid is designated as *heparin*[1] to indicate its origin from liver. It inhibits coagulation, partially or completely according to the concentration. Second, a substance present in blood plasma and blood serum which is converted into antithrombin by a reaction with heparin. Just as in plasma and serum there is a mother substance from which thrombin is formed, so there is an antecedent substance from which antithrombin is formed. By analogy this substance is designated as pro-antithrombin. Prothrombin is activated to thrombin by calcium, the pro-antithrombin is activated to antithrombin by heparin.

Preparation of heparin. Attention was first called to this substance during some work done in this laboratory by Jay McLean (2). In the course of his work McLean prepared cuorin from heart muscle by the method of Erlandsen (3) and attempted to prepare heparphosphatid from the liver by the method of Baskoff (4). The cuorin obtained corresponded in all of its properties and in its N to P ratio with the material described by Erlandsen. When freed from contamination with cephalin the cuorin in solution in sodium chloride, 0.9 per cent, was found to have an inhibiting action on clotting. The phosphatid obtained from the liver did not correspond in its properties or in its N to P ratio with the heparphosphatid described by Baskoff. When freed from mixture with cephalin, a result most difficult to accomplish, this substance had a very marked inhibiting effect upon coagulation of blood, its action in this respect being much stronger than that of cuorin. We have varied the method of preparation in many different ways in the hope of devising a rapid and economical process for the isolation of this inhibiting phosphatid. The method finally selected, while yielding a reliable preparation of heparin, is time-consuming and expensive in material. The main obstacle lies in the fact that the solubilities of heparin and cephalin are so similar that there is great difficulty in separating them. But until the cephalin or cephalin-like material is removed the properties of the heparin, so far as coagulation is concerned, are masked or antagonized. The method of preparation finally adopted is as follows:

---

[1] In a previous publication (Harvey Lectures, 1916-17, Series xii) this substance was described under the name of *Antiprothrombin*.

Fresh livers, preferably from dogs, are perfused, when possible, through the portal vein with a solution of sodium chloride, 0.9 per cent. They are then ground to a pulp, spread thin on a glass plate and dried in a current of hot air. The dried material is pulverized and extracted with ether in the proportion of 300 cc. of ether to each 100 grams of dried liver. The extraction may be made in a few hours with a shaking machine or by allowing the mixture to stand for 24 to 48 hours with an occasional shaking. The extract is then filtered and refiltered in a closed space until the filtrate is free from turbidity. The dark red filtrate is evaporated to dryness before an electric fan, and the sticky residue is treated with water-free ether (Squibb's) in as small quantities as suffice to dissolve it. There is always in this extract an insoluble residue which must be centrifugalized off. The clear red extract is then precipitated by acetone, the extract being added slowly with stirring to four times its volume of acetone. The precipitation is done in centrifugal tubes and the mixture is then centrifugalized. The supernatant liquid is drained off and discarded. The residue is dried in a current of air to drive off the adherent acetone. The residue is again dissolved in water-free ether, using as little ether as possible, and the solution is centrifugalized for a few minutes and poured off from any residue that may be obtained. The ether solution is then added slowly with stirring to four times its volume of absolute alcohol at 50°C. The mixture is kept in the warm bath for some time until the precipitate has settled. The supernatant liquid is decanted as far as possible and the balance is poured into centrifugal tubes and centrifugalized while warm. The supernatant liquid is poured off and the residue is dried in a current of warm air until the excess of alcohol is driven off. The residue is again dissolved in water-free ether, centrifugalized, precipitated at 50°C. by four volumes of absolute alcohol and so on as described above. This procedure is repeated until the precipitate formed by the absolute alcohol begins to lose its solubility in water-free ether. This change in solubility of the alcohol precipitate takes place quite suddenly sometimes after twelve to twenty precipitations, but sometimes more gradually. When, after at least two hours treatment with ether, the residue on centrifugalization is suddenly increased in amount, the looked-for change is taking place. Thereafter each such residue is preserved. It is first washed with fresh ether and is then dissolved in water-free chloroform (Squibb's). The chloroform solution of the material is given two final precipitations. First, it is precipitated by two volumes of absolute alcohol at 50°C. This precipitate is separated by centrifugalization and is again treated with chloroform. After the material is completely dissolved the solution is precipitated, this time at room temperature, by the addition of an equal volume of absolute alcohol. The precipitate is centrifugalized off and dried in a current of warm air. The dark residue is ground in a mortar to a chocolate colored powder and kept until needed in a desiccator. This material should be completely soluble in water giving, according to concentration, a clear brownish or red solution, with no indication of opalescence. Material kept now for two years shows no deterioration in its property of preventing coagulation.

Properties of heparin. As stated above the material is soluble in water without opalescence. The occurrence of opalescence in the solutions is an indication of admixture with cephalin and if much of the latter is present the inhibiting effect on coagulation is lessened or absent altogether in freshly made preparations. When such preparations are kept for some weeks or months it will be found that the inhibiting action of their solutions is greatly improved. The explanation of this change is found in the fact that cephalin exposed to air loses gradually its favoring action upon coagulation (5).

The mode of preparation of heparin indicates that it belongs to the group of phosphatids—it gives reactions for nitrogen and phosphorus. Through the kindness of Doctor Levene of the Rockefeller Institute analyses for N and P were made in two samples. As these samples were made a year apart the mode of preparation was slightly different in the two cases but both preparations gave clear, non-opalescent solutions which exhibited a very marked inhibiting effect upon coagulation and when added to plasma or serum caused the formation of antithrombin. The results of these analyses were as follows:

Preparation I—March, 1917

Nitrogen	5.01 per cent
Phosphorus	4.75 per cent
Ratio of nitrogen to phosphorus	2.4 to 1

Preparation II—March, 1918

Nitrogen	5.92 per cent
Phosphorus	4.93 per cent
Ratio of nitrogen to phosphorus	2.6 to 1

The preparations had therefore a fairly constant composition in nitrogen and phosphorus, the ratio of one to the other being probably 2.5 to 1.

As compared with the phosphatids that have been obtained from the liver, heparin resembles most some of the preparations of jecorin that have been described. For his preparations of jecorin, Baskoff gives a mean value of the N to P ratio of 2.38 to 1, but in the preparations of other workers this ratio has varied from 1.8 to 1 (Drechsel) to 6.08 to 1 (Siegfried and Mark). For his preparations of heparphosphatid, Baskoff gives the N to P ratio as approximately 1 to 1.5. The two characteristic reactions of heparin that are of special physiological interest are first, that it retards or prevents the coagulation of blood; and second, when added to plasma or serum it causes the formation of a notable amount of antithrombin. Both reactions have been studied as carefully as possible.

The inhibiting action on coagulation. This effect may be demonstrated on the shed blood or upon the circulating blood by intravascular injections. The solutions of heparin used were made up in 0.9 per cent sodium chloride to prevent hemolysis of the blood corpuscles. In shed blood it was found that in mixtures containing 0.1 per cent of heparin the coagulation was prevented entirely. In more dilute solutions the coagulation was delayed in proportion to the concentration. When injected intravenously kymographic records showed that there was no disturbance of heart rate, blood pressure or respiratory rhythm and no variation in rectal temperature. If the injection of heparin is made in the proportion of one decigram per kilogram of body weight, blood drawn within a few minutes after the injection shows no spontaneous coagulation. Later the effects of the injected heparin wear off gradually so that in three or four hours the coagulation time of the blood has returned to normal. The gradual wearing off of the effect of the injected heparin was shown also in the variations of the prothrombin time (6)—that is to say, the time of coagulation of the oxalated plasma when recalcified with the optimum amount of calcium chloride. Thus in one of the earlier experiments the following figures were obtained:

Prothrombin time before injection—dog	5 minutes
Prothrombin time 10 minutes after injection	No clot in 24 hours
Prothrombin time 1 hour after injection	38 minutes
Prothrombin time 2 hours after injection	14 minutes

Experiments made to determine how the heparin acts in preventing coagulation gave decisive proof that it inhibits the activation of prothrombin to thrombin. Its action certainly is entirely different from that of hirudin. This latter substance is a true antithrombin—that is to say, when added in minute amounts to mixtures of thrombin and fibrinogen it prevents clotting. Heparin has no such effect. Added in relatively large amounts to solutions of thrombin it has no retarding effect upon the action of the thrombin on fibrinogen. Since the heparin has no action upon fully formed thrombin and since it prevents the formation of thrombin when oxalated plasma is recalcified, it follows that the heparin must prevent the activation of prothrombin to thrombin, probably either by a reaction with the prothrombin or by a reaction with calcium. There are no facts to support the latter view. Heparin gives no precipitate with calcium salts and no indication of combining with the calcium in any way. Blood that has been rendered incoagulable with heparin cannot be made to clot by adding calcium chloride in any amounts. For example: in one experiment blood was caught as it flowed from an artery into a solution of heparin so that the concentration of the mixture was 0.15 per cent in heparin. This mixture was centrifugalized and the clear plasma was removed. This plasma remained unclotted as long as it was observed (24 hours). Mixtures were made with a solution of calcium chloride 0.5 per cent in the proportions of 5 drops of plasma to 1, 2, 3, 4 and 8 drops of the calcium chloride. None of these mixtures, or of similar mixtures in which the calcium chloride was replaced by water, showed any signs of clotting within 24 hours. The probability therefore is that the heparin reacts in some way with the prothrombin to prevent its activation by calcium. This conclusion is supported by direct experiments with solutions of prothrombin. Repeated experiments of this character were made with prothrombin prepared from the oxalated plasma of cat's blood (7). For example:

Prothrombin solution	5 drops
Heparin (0.2 per cent)	2 drops
Calcium chloride (0.5 per cent)	2 drops
Fibrinogen	8 drops

This mixture gave no clot in 24 hours—whereas a control mixture in which the heparin was replaced by an equal amount of water gave a solid clot within 5 minutes.

Heparin is a stable compound. As stated above, preparations kept in desiccators have retained their properties unimpaired for at least two years. Its solutions are not injured by heating to the boiling point. Specific experiments of this kind have been made with solutions containing heparin in concentrations of 0.5, 0.25 and 0.12 per cent. The test tubes containing these solutions were immersed for five minutes in boiling water. Subsequent examination showed that the activity of these solutions in preventing clotting and in activating pro-antithrombin had not been impaired.

The activation of pro-antithrombin. Early in this work it was observed that while heparin itself does not act as an antithrombin nevertheless it causes a marked increase in the antithrombin of plasma or serum. The following experiment may be used to illustrate this reaction. Clear oxalated and centrifugalized plasma from cat's blood was heated to 54°C. in a water bath to remove its fibrinogen. The precipitated fibrinogen was removed by filtration and the plasma was then tested for antithrombin by the method used in this laboratory (8) with and without the addition of a solution of heparin. One drop of each solution was added to 2, 3, 4 and 5 drops of thrombin and after an incubation of 15 minutes 10 drops of fibrinogen were added to each specimen and the time of coagulation was noted.

Specimen I. Plasma plus an equal volume of 0.1 per cent heparin

Thrombin 2 drops	No clot in 24 hours
Thrombin 3 drops	Clot in 95 minutes
Thrombin 4 drops	Clot in 42 minutes
Thrombin 5 drops	Clot in 17 minutes

Specimen II. Control—Plasma plus an equal volume of water

Thrombin 2 drops	Clot in 17 minutes
Thrombin 3 drops	Clot in 5 minutes
Thrombin 4 drops	Clot in 4 minutes
Thrombin 5 drops	Clot in 3 minutes

As will be seen, the specimen containing heparin showed a greatly increased amount of antithrombin. This experiment was repeated a great many times with specimens of serum as well as of plasma and always with similar results. In attempting to explain this reaction it was at first supposed that the heparin reacted with some of the known substances in serum to produce an antithrombin, for example, with prothrombin or antithrombin itself, or that in some way it sensitized or intensified the action of the antithrombin already present in the plasma or serum (9). Experiments made to test these hypotheses have given negative results. Prothrombin can be isolated in an impure but effective form by the acetone method. Solutions of this kind have no antithrombic action and addition of heparin does not alter the reaction in this respect. On the other hand it was found that the antithrombin in cat's plasma can be removed or destroyed by heating to 60°C. for five to ten minutes. Nevertheless on adding heparin to such a plasma there is an immediate production of a considerable amount of antithrombin. Hence the heparin does not act by intensifying the activity of antithrombin already present but produces new antithrombin by a reaction with an unknown constituent of the blood. This conclusion is strengthened further by the fact that this antecedent or mother substance can be precipitated from plasma by one-third or one-half saturation with ammonium sulphate. The precipitation by one-third saturation is less complete that with one-half saturation but in neither case is the active antithrombin thrown down, or if it is precipitated it is destroyed or denatured in the process. For example: a specimen of cat's plasma, oxalated and centrifugalized, was divided into two portions; to one portion ammonium sulphate was added to one-third saturation and to another to one-half saturation. The precipitates were collected by centrifugalizing and were then dissolved by the addition of water. Each solution was tested for antithrombin after adding an equal volume of heparin (0.2 per cent) and, as a control, after adding an equal volume of water, with the following result:

I. The precipitate obtained by one-third saturation with ammonium sulphate

With heparin

Thrombin 2 drops	No clot in 1 hour
Thrombin 3 drops	No clot in 1 hour
Thrombin 4 drops	Clot in 53 minutes
Thrombin 5 drops	Clot in 15 minutes
Thrombin 2 drops	No clot in 1 hour

With water (control)	
Thrombin 2 drops	Clot in 4.5 minutes
Thrombin 3 drops	Clot in 3.5 minutes
Thrombin 4 drops	Clot in 2.5 minutes
Thrombin 5 drops	Clot in 2.5 minutes

II. The precipitate obtained by one-half saturation with ammonium sulphate

With heparin	
Thrombin 2 drops	No clot within an hour
Thrombin 3 drops	No clot within an hour
Thrombin 4 drops	No clot within an hour
Thrombin 5 drops	No clot within an hour
With water (control)	
Thrombin 2 drops	Clot in 4 minutes
Thrombin 3 drops	Clot in 3 minutes
Thrombin 4 drops	Clot in 2.5 minutes
Thrombin 5 drops	Clot in 2 minutes

These results can scarcely be interpreted otherwise than upon the assumption that there is a material in plasma and serum which by a reaction with heparin is converted to or gives rise to antithrombin. In the nomenclature of the day we may speak of this substance as a pro-antithrombin which is activated to antithrombin by heparin, just as prothrombin is activated to thrombin by calcium.

The two sets of compounds show an interesting difference in their reactions to acetone. The precipitate obtained by adding an equal volume of acetone to blood plasma yields on appropriate treatment an active prothrombin but no pro-antithrombin. So also from solutions in which it is contained thrombin may be precipitated uninjured by acetone while antithrombin is destroyed or denatured. On the other hand ammonium sulphate to one-half saturation precipitates uninjured prothrombin, thrombin and pro-antithrombin, while antithrombin is either not precipitated or else is destroyed or denatured. It was observed also that in cat's oxalated plasma, after heating to 54°C. to remove the fibrinogen, both prothrombin and pro-antithrombin may be precipitated in part by the addition of acetic acid to distinct acid reaction. The precipitate may be collected by centrifugalizing and then dissolved in a solution of sodium chloride, 1 per cent, made slightly alkaline with sodium carbonate. This solution furnishes thrombin on the addition of calcium chloride and antithrombin on the addition of heparin.

The pro-antithrombin or antithrombogen, as it might also be called, is thermolabile but less so than antithrombin. As stated above, this difference is especially well shown in cat's plasma. In this plasma all the ready formed antithrombin may be destroyed by heating to 60° for 5 minutes while the pro-antithrombin is apparently not affected. Heating this plasma to 70°C. however will destroy all the pro-antithrombin very promptly, and the same result is obtained by prolonged heating at lower temperatures. The following results were obtained from the plasma of cat's blood, oxalated and centrifugalized, subsequently heated to 60° for 5 minutes and 15 minutes and to 70° for 5 minutes and then examined for antithrombin with and without the addition of heparin.

I. Specimen heated to 60°C. for 5 minutes

With heparin	
Thrombin 2 drops	No clot in 24 hours
Thrombin 3 drops	No clot in 24 hours
Thrombin 4 drops	No clot in 24 hours
Thrombin 5 drops	No clot in 24 hours
With water (control)	
Thrombin 2 drops	Clot in 4.5 minutes
Thrombin 3 drops	Clot in 3.5 minutes
Thrombin 4 drops	Clot in 3.5 minutes
Thrombin 5 drops	Clot in 3.5 minutes

II. Specimen heated to 60°C. for 15 minutes

With heparin	
Thrombin 2 drops	Clot in 32 minutes
Thrombin 3 drops	Clot in 10 minutes
Thrombin 4 drops	Clot in 10 minutes
Thrombin 5 drops	Clot in 5 minutes

III. Specimen heated to 70°C. for 5 minutes

With heparin	
Thrombin 2 drops	Clot in 4.5 minutes
Thrombin 3 drops	Clot in 3.5 minutes
Thrombin 4 drops	Clot in 2.5 minutes
Thrombin 5 drops	Clot in 2.5 minutes

It will be seen that the reaction with heparin was much weakened by heating to 60°C. for 15 minutes, and completely destroyed by heating to 70° for 5 minutes.

The source of pro-antithrombin. An effort was made to determine whether or not the pro-antithrombin is furnished to the plasma by the blood corpuscles. The experiments were not completed but they were carried far enough to show that neither the blood plates nor the leucocytes give origin to this substance. Platelets obtained from oxalated pig's blood by differential centrifugalization and subsequent washing with a 0.9 per cent solution of sodium chloride give a solution when extracted with water which contains prothrombin but no pro-antithrombin—that is to say, addition of calcium chloride causes the formation of thrombin, but addition of heparin does not produce antithrombin. A similar negative result, so far as pro-antithrombin is concerned, was obtained by extracting lymphocytes furnished by the thymus gland and blood leucocytes obtained from the buffy coat of centrifugalized oxalated blood.

The reaction between heparin and pro-antithrombin. We have no knowledge regarding the chemical nature of pro-antithrombin or the change that it undergoes in being converted to active antithrombin, but it seems evident that the reaction is not enzymatic or catalytic. The amount of antithrombin formed increases in proportion to the amount of heparin added. If one takes several specimens of oxalated plasma of 0.5 cc. each and adds one, five and ten drops of a 0.2 per cent solution of heparin it will be found that the amount of antithrombin formed increases with the heparin added. Unfortunately the mode of demonstrating antithrombin does not lend itself readily to a quantitative determination, except within narrow limits, so that it is not possible to express the relationship between the amounts of heparin and the amounts of antithrombin in a definite form. But the fact that the reaction between the heparin and the pro-antithrombin is of the nature of a definite chemical reaction is borne out also by the following observation. A specimen of clear cat's plasma, oxalated, was heated to 60°C. for ten minutes, thereby precipitating its fibrinogen and destroying all the antithrombin present. After filtration, a 0.2 per cent solution of heparin was added in equal volume to a part of this plasma. Examination showed now the presence of a considerable amount of antithrombin, due to the action of the heparin on the pro-antithrombin. This mixture was then heated again to 60°C. for ten minutes to destroy the newly formed antithrombin, and that this really occurred was determined by experiment. To the mixture there was again added an equal volume of heparin, 0.2 per cent, with the result that there was again a formation of a considerable amount of antithrombin. The experiment serves not only to indicate that the reaction between the heparin and pro-antithrombin takes place in definite proportions after the manner of an ordinary chemical reaction, but it serves to show also that the reserve supply of pro-antithrombin in blood is considerable. The reaction between antithrombin and thrombin, as has been pointed out previously (10), is a colloidal reaction the extent of which varies with the time, so that in any effort to estimate the amount of antithrombin in a quantitative way in different solutions it is necessary to give a definite time for the interaction between the two substances. The longer they are in contact the larger will be the amount of thrombin inactivated by the antithrombin. The relations are quite different in the reaction between heparin and pro-antithrombin. When a mixture is made the maximal reaction is completed as soon as an examination can be made, and a longer exposure leads to no further increase in antithrombin.

DISCUSSION

One, at least, of these new factors in coagulation, the pro-antithrombin, is present normally in blood. The other, the heparin, has not been shown directly to be a normal constituent of blood but the indirect evidence speaks strongly in favor of this view. The direct rôle of the pro-antithrombin is to give rise to antithrombin and it may be assumed that the amount of the latter constantly present in normal blood is dependent upon the extent to which the pro-antithrombin is activated, and this in turn is dependent upon the amount of heparin since so far as we know the pro-antithrombin cannot be changed to antithrombin otherwise than by a reaction with heparin. The fact that there is a supply, and a considerable supply, of pro-antithrombin in circulating blood indicates that the antithrombin probably takes an important part in regulating the process of coagulation. What is the rôle of antithrombin? Two views have been suggested: Nolf (11) and afterwards Howell (12) have stated their belief that the antithrombin is directly concerned in maintaining the normal fluidity of the blood. The latter in his theory of coagulation has assumed that some inhibiting substance, presumably the antithrombin, functions by preventing the activation of prothrombin by calcium and that in this way intravascular coagulation is avoided. When blood is shed or when in any other way thromboplastic substance is added to the blood, the inhibiting influence of the antithrombin is neutralized, the prothrombin is converted to thrombin and coagulation ensues. At the time this theory was proposed antithrombin was the only inhibiting substance known in the blood, but the work described in this paper makes it probable that the blood contains a second inhibiting substance, heparin, which so far as its action is concerned, may be designated as an anti-prothrombin since it inhibits clotting by preventing the activation of prothrombin. It would be more logical perhaps to assume that heparin rather than antithrombin is the substance in blood which prevents spontaneous clotting and which is neutralized in shed blood by the thromboplastic material, the cephalin. Laboratory experiments show readily that the inhibiting effect of heparin can be neutralized by the addition of cephalin, but in order to give this modification of Howell's original theory a sound experimental basis it is necessary to show that heparin is a normal constituent of blood. This demonstration has not yet been furnished. But admitting the probability of this view the question arises as to the function fulfilled by the antithrombin which we know does exist in normal blood. In several pieces of work done in this laboratory (Weymouth, 13), (Gasser, 14), (Rich, 15), evidence has been furnished which tends to show that metathrombin found in the serum of clotted blood is a combination of thrombin and antithrombin. The antithrombin inactivates thrombin by forming the substance designated as metathrombin by Morawitz. In accordance with this view Gasser has suggested that this may be at least one function of the antithrombin. It is very probable that thrombin in small amounts is formed constantly in circulating blood and the presence of antithrombin may be a device to neutralize this thrombin. On the other hand, when blood is shed thrombin is formed suddenly in relatively large amounts and the antithrombin under such conditions is inadequate for protection, especially as its rate of action upon thrombin is greatly reduced at temperatures below that of the body (16). Provisionally we are inclined to adopt the view that the value of the antithrombin and therefore of the pro-antithrombin is to safeguard the blood from the action of any free thrombin that may be liberated in the circulation.

The function of the heparin in coagulation is implied in its two characteristic properties described above. As previously stated, the presence of this substance in the blood has not been demonstrated but it seems very probable that it is one of the normal constituents of this liquid. The fact that it has the specific reaction of changing pro-antithrombin to antithrombin supports this view. If we can make this assumption it would follow that heparin constitutes an inhibiting material which restrains clotting by a double action; by its power to prevent the activation of prothrombin to thrombin and by its property of activating pro-antithrombin to antithrombin. Through this material the body is able to modify coagulability of the blood either locally or in general. An instance of local modification is furnished by the work of Schickele (17) who has shown that an inhibiting substance to blood coagulation is produced in the uterine mucous membrane. A special study of this material in this laboratory by Dr. Jessie King has shown that it is undoubtedly heparin or a substance with properties similar to those of heparin, since it inhibits clotting by preventing the activation of prothrombin and is also effective in causing an increase in antithrombin when added to plasma or serum. It is quite possible indeed that some of the normal and abnormal variations in the coagulability of blood may be traced to variations in the amount of heparin. The properties of hemophilic blood, for example, as described by one of us (18), are in fact identical with those exhibited by blood to which heparin has been added in concentrations sufficient to retard coagulation. If subsequent work is successful in devising a method for the quantitative estimation of heparin in blood, a great amount of light may be thrown upon some of the difficult problems in blood coagulation.

SUMMARY

1. Two new substances are described which are connected with the process of coagulation of blood:

a. A phosphatid designated as heparin since it is obtained most readily from the liver (dog). It has an N to P ratio of 2.5 to 1. It is easily soluble in water giving a clear, non-opalescent solution of a yellowish or reddish color according to concentration. It possesses two characteristic reactions: First, it retards or prevents the coagulation of blood, both in the body and when the blood is shed. Second, it causes a marked increase in antithrombin when added to blood or serum.

b. An antecedent or mother substance for antithrombin, present in plasma and serum in considerable amounts. It is converted promptly to antithrombin by heparin and is designated as a pro-antithrombin.

2. Heparin may be obtained from lymph glands and it has been detected by its reactions in the uterine mucous membrane. It is present in largest amounts in the liver, especially the dog's liver.

3. Heparin inhibits clotting mainly by preventing the activation of prothrombin to thrombin. It acts as an antiprothrombin rather than as an antithrombin.

4. The action of heparin in preventing the activation of prothrombin and in causing the activation of pro-antithrombin is not destroyed at a temperature of 100°C.

5. Pro-antithrombin in blood plasma is destroyed promptly by heating to 70°C. and more gradually by prolonged heating at lower temperatures (60°C.).

6. Pro-antithrombin may be precipitated uninjured from blood plasma by acetic acid or by ammonium sulphate added to one-half saturation.

7. On the theoretical side it is suggested that heparin and pro-antithrombin are normal constituents of the circulating blood (not demonstrated for the heparin) and together fulfill the function of safeguarding the fluidity of the blood, that is to say, of preventing intravascular clotting. The pro-antithrombin by its conversion to antithrombin provides a protection against any small quantities of thrombin that may arise in the circulating blood (metathrombin formation). The heparin in addition to functioning as a specific activator to pro-antithrombin exerts an inhibiting influence upon the conversion of prothrombin to active thrombin. When blood is shed or when in other ways thromboplastic substance (cephalin) is added to the blood the protection afforded by the heparin is overcome and thrombin is formed in amounts sufficient to cause clotting. It is suggested that variations in the amount of heparin in the blood may suffice to explain some of the known abnormalities in coagulation, hemophilia, for example.

BIBLIOGRAPHY

(1) Howell: This Journal, 1912, xxxi, 1.
(2) McLean: Ibid., 1916, xli, 250.
(3) Erlandsen: Zeitschr. f. physiol. Chemie, 1907, li, 71.
(4) Baskoff: Zeitschr. f. physiol. Chemie, 1908, lvii, 395.
(5) McLean: This Journal, 1917, xliii, 586.
(6) Howell: Arch. Int. Med., 1914, xiii, 76.
(7) Howell: This Journal, 1914, xxxv, 474; also The Harvey Lectures, Series xii, 1916–17.
(8) Howell: Arch. Int. Med., 1914, xiii, 76; also The Harvey Lectures, Series xii, 1916–17 for modification.
(9) Howell: The Harvey Lectures, Series xii, 1916–17, 317.
(10) Howell: Ibid., 302.
(11) Nolf: Arch. d. Fisiol. 1909, vii, 1.
(12) Howell: This Journal, 1911, xxix, 187.
(13) Weymouth: Ibid., 1913, xxxii, 266.
(14) Gasser: Ibid., 1917, xlii, 378.
(15) Rich: Ibid., 1917, xliii, 549.
(16) Howell: Ibid., 1914, xxxvi, 1.
(17) Schickele: Biochem. Zeitschr., 1912, xxxviii, 169.
(18) Howell: Arch. Int. Med., 1914, xiii, 76.

THE INFLUENCE OF SECRETIN ON THE NUMBER OF RED AND WHITE CORPUSCLES AND THE FERMENTS AND SUGAR CONTENT OF THE BLOOD

B. FUJIMOTO

From the Forensic-Medical Institute of the Imperial University at Tokyo

Received for publication October 17, 1918

In 1902 Bayliss and Starling (1) showed that acid acts on prosecretin in the duodenal mucous membrane and changes it into secretin, which is carried by the blood and activates the pancreatic cells. They showed also that secretin increases the secretion of bile.

But as to its effects on the internal secretion no researches have apparently been carried out, except the fairly recent work of Downs and Eddy, (2), (3) who performed several interesting experiments and found that the subcutaneous injection of even a small dose of secretin is able to produce a marked increase in the number of red and white corpuscles in the circulating blood. They suggested as the most probable explanation of this increase that it is due to a direct stimulating action of the secretin on the bone marrow and lymphatic tissues in general.

In our laboratory more extensive researches in this field have been carried out, namely: the influence of secretin on several ferments (catalase, diastase and glycolytic ferment) and on the sugar content of the blood.

The secretin which we used was always prepared as follows: Dogs weighing about 10 kgm. each were used. They received no food for about twenty-four hours previous to being bled. The mucous membrane was scraped off with a dull knife from the duodenum and the upper half of the small intestine, and was triturated with 50 cc. of 0.4 per cent hydrochloric acid. After standing for two hours, the mixture was boiled actively, neutralized with 10 per cent solution of sodium hydroxide while boiling and again rendered faintly acid with glacial acetic acid. After boiling for about ten minutes the preparation was filtered. The filtrate thus obtained was clear and greenish-yellow. Sufficient glacial acetic acid was added to make it 2 per cent by volume and the acid extract evaporated to dryness, as was done by Downs and Eddy. We thus obtained about 0.5 to 0.7 gram of dried acid extract, namely about 10 to 14 mgm. per cubic centimeter of original solution.

In our experiments the dried acid extract was dissolved in normal saline solution and injected into rabbits subcutaneously. In all experiments recorded in this paper rabbits were employed. During the experiments food and water were always withheld from the animals.

The investigations to be described were commenced with the repetition of Downs and Eddy's work.

The influence of secretin on the number of red and white corpuscles in the circulating blood. Downs and Eddy have shown that the administration of secretin produces a considerable increase in both the red and the white corpuscle count. Our experiments were carried out in accordance with their procedure. The blood was obtained from the ear of the rabbit. Specimens were taken simultaneously for counting both red and white corpuscles, Hayem's solution being used as the diluting fluid for the former and Türk's solution for the latter. The counts were made in the usual manner with the Thoma-Zeiss apparatus.

One cubic centimeter of the secretin solution, equivalent to approximately 10 mgm. of the dried acid extract, per kilogram of body weight, which was determined by Downs and Eddy as the most efficient dose to produce a marked increase in the number of red and white corpuscles per cubic millimeter of the circulating blood, was injected subcutaneously. The results are shown in table 1.

These experiments show that the secretin solution, when injected subcutaneously, is able to produce an increase in the number of red and white corpuscles in the circulating blood thus confirming the results of Downs and Eddy.

The influence of secretin on the catalase content of the blood. The object of this investigation was to determine what change, if any, occurs in the catalase content of the blood. The amount of catalase in the blood was determined according to the following method.

The blood was obtained in all cases from the ear of the rabbit with as little manipulation as possible, and quickly defibrinated. One cubic centimeter of this defibrinated blood was diluted with normal saline solution to 0.1 per cent. Ten cubic centimeters of this solution of the blood were added to 30 cc. of 1 per cent neutral hydrogen peroxide in a specially arranged flask. The liberated oxygen gas was conducted through a rubber tube to a graduated burette, which was previously filled with water. As the liberation of the oxygen is considerably accelerated by shaking the flask and therefore the amount of the liberated gas may vary with such shaking, we have, as a rule, never touched the apparatus after the blood specimen and hydrogen peroxide were mixed. Exactly after twenty minutes, the atmospheric pressure in the burette was made equal to that in the room and the amount of the oxygen was read off directly from the burette in which the gas had displaced the water. The volume of oxygen gas which is liberated by 1 cc. of the blood in a minute was taken as a measure of the amount of catalase in the defibrinated blood.

TABLE 1

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER		INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN
						minutes
1	R. B. C.	5,550,000	7,030,000	1,480,000	27	60
	W. B. C.	10,100	10,700	600	6	60
2	R. B. C.	5,110,000	6,780,000	1,670,000	33	90
	W. B. C.	15,200	23,300	8,100	19	90
3	R. B. C.	5,870,000	7,030,000	1,160,000	20	60
	W. B. C.	6,100	9,000	2,900	38	60
4	R. B. C.	5,720,000	7,640,000	1,920,000	34	90
	W. B. C.	11,000	16,200	5,200	47	120
5	R. B. C.	6,130,000	10,070,000	3,940,000	64	90
	W. B. C.	5,200	10,200	5,000	96	90
6	R. B. C.	6,060,000	6,970,000	910,000	15	90
	W. B. C.	6,400	8,500	2,100	33	90
7	R. B. C.	5,940,000	6,420,000	460,000	8	90
	W. B. C.	9,000	11,300	2,300	26	90
8	R. B. C.	6,240,000	6,560,000	320,000	5	90
	W. B. C.	7,900	8,100	200	3	90
Average percentage increase	R. B. C.					25.75
	W. B. C.					34.75

Burge (4), (5) has reported the effect of the emotions on the catalase content of the liver and blood. He has also shown that the catalase content of the blood is increased by prolonging the excitement stage of ether anaesthesia and believes that the increase in catalase may account for the increased oxidation during the excitement stage of ether anaesthesia just as the decrease in catalase may account for the decreased oxidation during the subsequent stages of anaesthesia. Burge and Neill (6) also demonstrated that the catalase of the blood is decreased in the condition of "shock." In our preliminary experiments dealing with the effect of secretin on the blood catalase in rabbits it was found that the injection caused an actual decrease in the catalase content. A similar decrease was, however, noted in control animals. Subsequently it was discovered, as is indicated in table 2, that this decrease was apparently associated with the handling and fright of the animals for if time was allowed to elapse, the catalase determinations became reasonably constant.

TABLE 2

The catalase content of normal rabbits

RABBIT NUMBER	DATE	CATALASE					
		In the beginning	After				
			30 minutes	60 minutes	90 minutes	120 minutes	150 minutes
6	May 22	67.0	45.0	41.0	37.0		
7	May 23	55.0		52.5	45.0	44.0	44.0
8	May 24	100.0	95.5	89.5	87.0	84.0	82.5
9	May 24	33.0	31.5	30.0	28.5	30.5	
6*	May 25	29.0		48.0	38.5	34.5	
1*	May 25	73.0		80.0	80.0	73.0	
10	May 27	90.0		87.0	89.0	86.5	86.0
11*	May 27	82.0		86.0		74.5	65.0
7	May 28	71.5		65.5	65.5	75.0	72.5
8*	May 28	71.5		74.0	73.0	77.0	

Four experiments marked with an asterisk (*) show an increase in the catalase content at first. Probably these animals were less frightened by repeating the experiment. As we see in the foregoing table, the catalase content in the blood decreases mostly during the beginning one and a half hour of the experiments and then becomes relatively constant.

In our experiments secretin was, therefore, injected hypodermically after the catalase content became relatively constant. One cubic centimeter of secretin per kilogram of body weight was given hypodermically. The results obtained will be found in table 3.

Experiments 4 and 9 showed no increase in the catalase content. In experiment 1 the secretin was injected intravenously. Except for these experiments, a dose of 1 cc. of secretin solution per kilogram of body weight was able to produce an average increase of 17 per cent in the catalase content of the circulating blood. It was not determined whether there is a more effective dose than the one used.

Several experiments for control were undertaken in order to determine if this increase in the catalase content of the blood is caused especially by the injection of secretin solution. At first, 2 or 3 cc. of normal saline solution were injected subcutaneously in rabbits, after the catalase content in the blood became relatively constant. But no increase in the catalase was seen, as the following table shows.

TABLE 3

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	RABBIT NUMBER	DATE	CATALASE						
			Before	Time after the injection of secretin					
				30 minutes	60 minutes	90 minutes	120 minutes	Percentage increase	Maximum in
									minutes
1	7	May 23	43.5	47.0	43.5	39.5	44.5	8	30
2	8	May 24	67.0	80.5	80.0	70.5	68.5	20	30
3	9	May 24	30.0	34.5	31.0	29.5	27.5	15	30
4	6	May 25	45.0	41.0	42.0	36.5		0	
5	1	May 25	53.0	71.0	80.0	67.0		50	60
6	10	May 27	81.0	83.0	88.0	90.0	76.5	11	90
7	11	May 27	69.0	72.0	80.0	73.0	69.5	11	60
8	8	May 28	77.0	81.0	75.0	76.0	70.0	5	30
9	7	May 28	75.0	72.5	66.5	64.0	68.5	0	

Average percentage increase, 17; maximum in 47 minutes

As the secretin solution contains peptone, we have proceeded to the further experiments for control in order to determine if the peptone itself has an effect on the catalase content of the blood. For this purpose we performed the following four experiments with a dose of 0.01 gram per kilogram of body weight. The peptone was dissolved in normal saline solution and injected hypodermically The results are shown in table 5.

A slight increase was found in two experiments. But comparing these results with those of the experiments with secretin, the increase is insignificant. Hence the conclusion is justified that the hypodermic injection of the secretin solution causes a considerable increase in the catalase content of the blood in thirty to ninety minutes. Since catalase is found in the blood corpuscles, it is natural to infer a relationship between the increased corpuscle count and the increase of catalase produced by the injection of secretin.

TABLE 4

Influence of the hypodermic injection of normal saline solution (2 or 3 cc.) on the catalase content in the blood

EXPERIMENT NUMBER	CATALASE			
	Before the injection	Time after the injection		
		30 minutes	60 minutes	90 minutes
1	34.5	32.5	32.0	31.0
2	44.0	44.0	44.5	43.5
3	77.5	80.5	71.0	67.0
4	33.0	32.0	30.5	30.0

TABLE 5

Experiments on the effect of peptone on the catalase content of the blood. Dose: peptone 0.01 gram per kilogram of body weight

EXPERIMENT NUMBER	CATALASE				
	Before the injection	Time after the injection			
		30 minutes	60 minutes	90 minutes	120 minutes
1	42.0	43.5	46.0	45.0	41.0
2	73.0	70.0	72.0	72.5	67.5
3	86.0	80.0	82.5	81.5	77.5
4	73.0	77.0	77.0	80.0	79.0

The influence of secretin on the diastase of the blood. Investigations were carried out to study the influence of secretin on several ferments in the blood. Diastase was first examined.

Inoue has recently made an interesting report on methods for the determination of diastase, which is not yet published. It was proved by him that Wohlgemuth's series method for the determination of diastase does not give reliable results. He has recommended a new method, which gives uniformly good results. In our experiments this new method was employed; namely: normal saline solution, which contains a certain quantity of salt and acetic acid, was always used for the dilution of the blood serum. One cubic centimeter of the diluted serum solution (in our cases 5 per cent) was added to 1 cc. of 0.1 per cent amylum solution and incubated in a water bath at 38°C. At intervals of a minute a drop of Lugol's solution (1/50 N) was added to it to see whether the amylum was completely digested or not. The time which is necessary for the complete digestion of the amylum was taken as a measure of the amount of diastase. The results here recorded are based upon the examination of twelve different specimens obtained from eight different rabbits.

TABLE 6

EXPERIMENT NUMBER	THE TIME WHICH IS NEEDED TO DIGEST 1 CC. OF 0.1 PER CENT AMYLUM SOLUTION COMPLETELY BY 1 CC. OF 5 PER CENT SERUM SOLUTION				
	Before the injection	Time after the injection of secretin, 1 cc. per kilogram of body weight			
		30 minutes	60 minutes	90 minutes	120 minutes
	minutes	minutes	minutes	minutes	minutes
1	24	24	24	25	26
2	26	27	28	30	31
3	25	28	31	33	30
Control: nothing injected					
1	26	26	29	28	27
2	27	28	28	30	31

These experiments show that the content of diastase in the blood is not influenced at all by the hypodermic injection of the secretin solution in a dose of 1 cc. per kilogram of body weight. Incidentally we tested the serum of these rabbits, in which secretin had been injected, on the enucleated eyes of frogs to see if there was any adrenalin-like substance in the blood. The results were entirely negative.

The influence of secretin upon the sugar and the glycolytic ferment of the blood. The blood was taken from the ear of rabbits and quickly defibrinated. Four cubic centimeters were added immediately to 36 cc. normal saline solution, containing a known quantity of 6 per cent solution of pure dextrose. Twenty cubic centimeters of the mixture were examined immediately, and the remainder was placed in an incubator at 37°C. for twenty hours. The blood sugar content was determined with the remaining portion of the defibrinated blood. Throughout the research rigid precautions as regards asepsis were taken.

The specimens were always precipitated by liq. ferri dialysat. and potassium sulphate (saturated solution), and filtered. In all estimations of sugar Pavy-Kumagawa-Sudo's method was employed. At first, 1 cc. of secretin solution per kilogram of body weight was given subcutaneously to see if it influenced the sugar and glycolytic ferment content of the blood. But, no influence being observed, 10 cc. per kilogram of body weight were administered. The results are shown in the following tables.

TABLE 7

EXPERIMENT NUMBER	SECRETIN	BLOOD SUGAR	
		Before the injection of secretin	One hour after the injection of secretin
	cc.	*per cent*	*per cent*
1	1	0.117	0.132
2	1	0.124	0.135
3	10	0.165	0.198
4	10	0.108	0.125
5	10	0.105	0.136

TABLE 8

EXPERIMENT NUMBER	SECRETIN		DEXTROSE			
			Immediately after	20 hours after at 36°C.	Loss of dextrose	Percentage loss
	cc.		*mgm.*	*mgm.*	*mgm.*	
1	1	Before injection	7.908	6.136	1.772	22.4
		One hour after	7.880	6.084	1.796	22.8
2	1	Before injection	8.020	5.156	2.864	35.7
		One hour after	8.020	5.342	2.678	33.4
3	10	Before injection	9.448	6.288	3.160	33.5
		One hour after	9.188	6.700	2.488	27.1
4	10	Before injection	8.772	5.460	3.312	37.8
		One hour after	8.732	5.648	3.084	35.3
5	10	Before injection	8.548	4.216	4.332	50.7
		One hour after	8.396	4.076	4.320	50.2

All of these experiments show some increase in the amount of the blood sugar. But, as the blood sugar content is labile, such a slight increase may be caused by factors such as excitement of the animal. It thus appears that the blood sugar is not influenced by the administration of secretin.

As we see in the foregoing table, no marked decrease in the glycolytic power of the blood was proved. It would thus appear that the sugar and glycolytic ferment of the blood are in no way altered quantitatively by the administration of secretin.

CONCLUSIONS

1. Secretin, when injected subcutaneously, produces an increase in the number of red and white corpuscles in the circulating blood of rabbits, as Downs and Eddy proved.
2. The hypodermic injection of 1 cc. of secretin causes an increase in the catalase content of the blood.
3. Neither increase nor decrease in the diastase, glycolytic ferment and sugar of the blood was observed after the hypodermic injection of 1 cc. of secretin.

I desire to express my sincere thanks to Prof. Dr. K. Katayama and Prof. Dr. S. Mita, to whom I am indebted for suggesting the problem and for their kind advice.

BIBLIOGRAPHY

(1) Bayliss and Starling: Journ. Physiol., 1902, xxviii, 325.
(2) Downs and Eddy: This Journal, 1917, xliii, 415.
(3) Downs and Eddy: This Journal, 1918, xlv, 294.
(4) Burge: This Journal, 1917, xliv, 75.
(5) Burge: This Journal, 1917, xliv, 290.
(6) Burge and Neill: This Journal, 1918, xlv, 286.

THE INFLUENCE OF THE INGESTION OF THE AMINO-ACIDS, ADEQUATE AND INADEQUATE PROTEINS UPON THE PRODUCTION OF CATALASE

W. E. BURGE

From the Physiological Laboratory of the University of Illinois

Received for publication October 19, 1918

Lusk (1) found that the ingestion of glycocoll, alanin, leucin and tyrosin increased oxidation while the ingestion of glutamic acid did not. Grafe (2), contrary to Lusk, found that the ingestion of glutamic acid as well as glycocoll, alanin, leucin and tyrosin increased oxidation. We (3) had found that whatever increased oxidation in the body produced a corresponding increase in catalase by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme, and that whatever decreased oxidation produced a corresponding decrease in catalase by decreasing the output from the liver and by the direct destruction of the enzyme in the blood and tissues. The object of the present investigation was to determine if the ingestion of amino-acids such as glycocoll and alanin would produce an increase in catalase parallel with the increase produced in oxidation, and whether amino-acids such as glutamic and aspartic would produce no increase in catalase in keeping with Lusk's contention that they do not increase oxidation, or whether they would produce an increase in catalase in keeping with Grafe's contention that the ingestion of these two amino-acids does increase oxidation. The effect of the ingestion of an adequate and of an inadequate protein was also studied. The adequate protein used was casein and the inadequate one, gelatin. A digest of each of these proteins was made by adding 200 cc. of activated pancreatic juice to 300 grams of each of the proteins in a small amount of a 1 per cent sodium carbonate solution. This material was kept in a thermostat at 40°C. for 200 hours. Thymol was the preservative used. The amounts of the amino-acids used were 5 grams per kilogram of body weight of the animal. The glutamic and aspartic acids were given in the form of the sodium salts and in concentrated solutions. The glycocoll and alanin were given in as concentrated solutions as could be made with distilled water. The whole of the 300 grams of the protein digests was used.

After etherizing the animals and opening the abdominal wall, the materials were introduced into the intestine under pressure from a bottle through a rubber tube and an attached hypodermic needle. The catalase in 0.5 cc. of blood taken directly from the liver, the portal and jugular veins was determined before as well as at fifteen-minute intervals after the introduction of the different materials. The blood of the liver was collected from a superficial incision made in this organ. The catalase was determined by adding 0.5 cc. of blood to 50 cc. of diluted hydrogen peroxide in a bottle at approximately 22°C., and the amount of gas liberated in ten minutes was taken as a measure of the amount of catalase in the 0.5 cc. of blood.

In figure 1 under glycocoll, alanin, glutamic acid, aspartic acid, asparagin, (glutamic acid, glycocoll), (aspartic acid, glycocoll), (asparagin, glycocoll), are given curves, constructed from data obtained from dogs before as well as at fifteen-minute intervals after the introduction of these materials into the alimentary tract. The figures along the ordinate represent amounts of catalase measured in cubic centimeters of oxygen and the figures along the abscissae, time in minutes. The continuous line curves were constructed from data obtained from the blood of the liver, the discontinuous line curves from the blood of the portal vein, and the dotted line curves from the blood of the jugular. It may be seen in figure 1, dog 1, under glycocoll, that previous to the introduction of the glycocoll, 0.5 cc. of blood from the liver liberated 84 cc. of oxygen from hydrogen peroxide while 0.5 cc. of blood from the portal and jugular veins liberated 64 and 66 cc. respectively. After the introduction of the glycocoll the catalase of the blood of the liver, portal and jugular veins was increased as is indicated by the increase in the amount of oxygen liberated from the hydrogen peroxide. The catalase of the blood of the liver was increased, particularly during the first fifteen minutes, more rapidly than that of the portal or jugular veins. This is taken to mean that the glycocoll was stimulating the liver to an increased amount of catalase. That the liver is continuously replenishing the blood and hence the tissues with catalase is shown by the fact that the blood of the liver is always richer in catalase by 15 to 20 per cent than the blood from any other part of the body. The fact also, as shown in a previous publication, that the introduction of a substance, such as ethyl alcohol, which normally produces a great increase in catalase, produced a very small or no in-

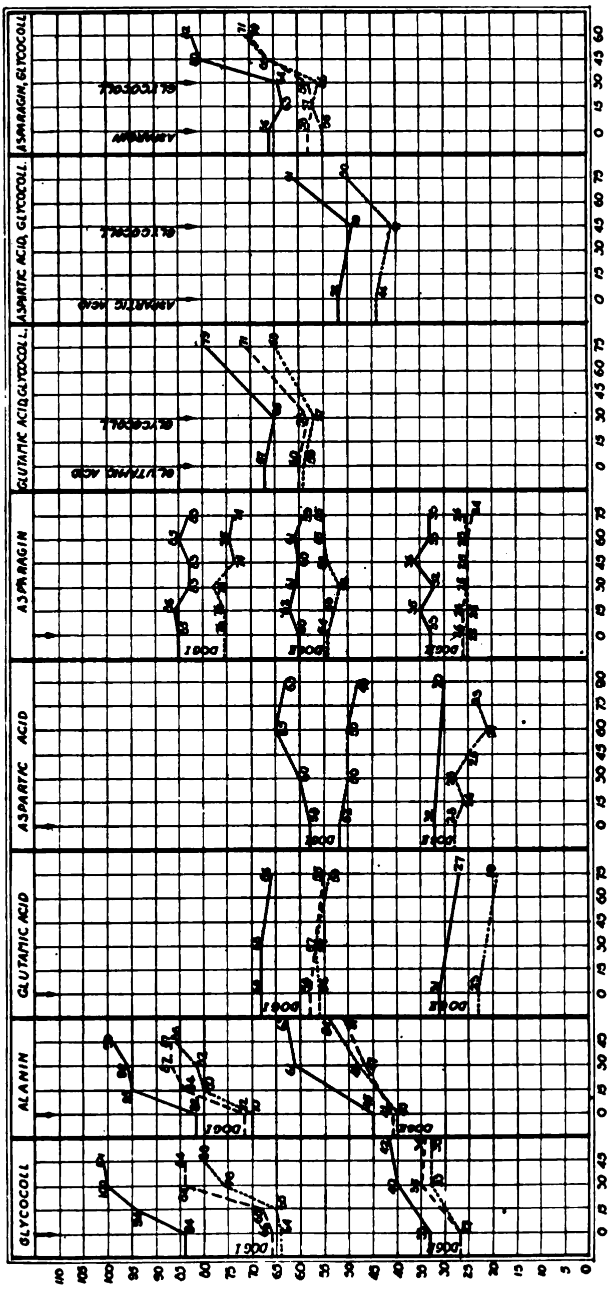


Fig. 1. Curves showing the effect on the production of catalase of the introduction into the intestines of glycocoll, alanin, glutamic acid, aspartic acid, asparagin, glutamic acid followed by glycocoll, aspartic acid followed by glycocoll, and asparagin followed by glycocoll. Continuous line curves show amount of catalase in the blood of the liver; discontinuous line curves show amount of catalase in the blood of the portal vein; dotted line curves show amount of catalase in the blood of the jugular. The figures along the abscissa indicate time in minutes and those along the ordinate, amounts of catalase measured in cubic centimeters of oxygen.

crease after the liver was cut out of the circulation by an Eck fistula and by ligating the hepatic artery was taken to mean that the liver is the organ principally responsible for the production of catalase. It may be seen that the alanin also produced an increase in catalase while the glutamic and aspartic acids and asparagin did not. It may also be seen under (glutamic acid, glycocoll), (aspartic acid, glycocoll) and (asparagin, glycocoll) that after the introduction of aspartic and glutamic acids and asparagin had produced no increase in catalase, the introduction of glycocoll produced a very marked increase.

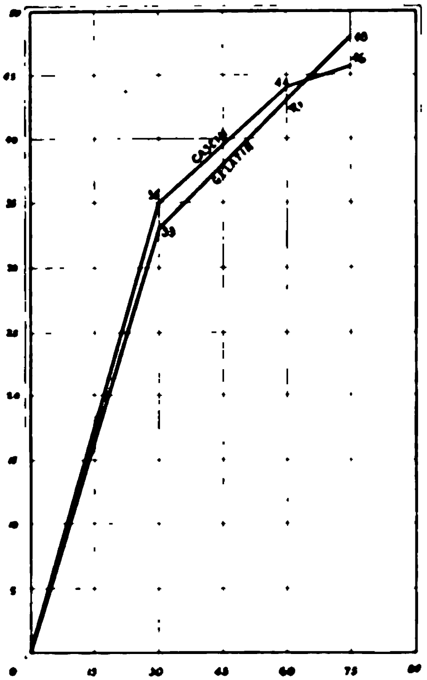


Fig. 2. Curves showing the effect of the introduction into the intestines of a pancreatic digest of an adequate protein, casein, and of an inadequate protein gelatin. The figures (0-80) along the abscissa indicate time in minutes, and the figures (0-50) along the ordinate, percentage increase in the catalase of the blood

In figure 2 the curves were constructed from data obtained before as well as at fifteen-minute intervals after the introduction into the intestines of a pancreatic digest of 300 grams of casein and of 300 grams of gelatin. The digest of each of these proteins was prepared and introduced as already described. It will be seen that the introduction of the digests of the adequate protein, casein, as well as of the inadequate protein, gelatin, increased the catalase of the blood very extensively, in keeping with the fact that the ingestion of these proteins increases oxidation.

SUMMARY

The ingestion of amino-acids such as glycocoll and alanin produces an increase in catalase with resulting increase in oxidation, by stimulating the digestive glands, particularly the liver, to an increased output of this enzyme; while the ingestion of amino-acids such as glutamic and aspartic produces no increase in catalase, in keeping with the fact that they cause no increase in oxidation.

The introduction of the digest of the adequate protein, casein, as well as the digest of the inadequate protein, gelatin, produces an increase in the catalase of the blood, in keeping with the fact that the ingestion of these proteins brings about an increase in oxidation.

BIBLIOGRAPHY

(1) **Lusk**: Journ. Biol. Chem., 1912, xiii, 155.
(2) **Grafe**: Deutsch. Arch. f. Klin. Med., 1915, cxviii, 1.
(3) **Kennedy and Burge**: Arch. Int. Med., 1917, xx, 892.
Burge and Neill: This Journal, 1918, xlvii, 13.
Burge: Science, N. S., 1918, xlviii, 1239, 327.

REGENERATION OF BLOOD SERUM PROTEINS

I. INFLUENCE OF FASTING UPON CURVE OF PROTEIN REGENERATION FOLLOWING PLASMA DEPLETION

WM. J. KERR,[1] S. H. HURWITZ AND G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication October 21, 1918

The experiments tabulated below were performed to obtain information concerning the regeneration and source of blood proteins, their fluctuation under abnormal conditions and their function in the body economy. The experimental evidence is sufficient to show that the regeneration of blood serum proteins is quite slow, in fact a matter of several days, following a marked depletion of blood proteins. This is in striking contrast to the rapid regeneration of fibrinogen (one of the blood plasma proteins), which has been studied previously by Whipple (1) and Goodpasture (2). Regeneration of the blood serum proteins can take place during starvation, although not as rapidly as on a full diet, but this shows that the body can manufacture these proteins at the expense of other body proteins or perhaps by conserving the amino acids which result from the protein katabolism of starvation.

It is not necessary to review the rather extensive literature on this subject as excellent reviews by Morawitz (3) in 1909 and Rowe (4) in 1916 are available. It is to be noted that all the earlier workers failed to demonstrate any constant fluctuations in the blood proteins during periods of fasting or after heavy feeding. Some observers noted a relative increase in globulins at the expense of albumins after long periods of fasting and others deny that these fluctuations are in excess of those noted in normal animals under the usual experimental conditions. We can agree with the general opinion that the blood serum proteins show but minimal fluctuations during periods of fasting or after heavy feeding and such fluctuations may be observed in normal animals (compare table 1). Our observations go to show that the amounts of blood proteins in dogs may vary more in different animals than in the same animal. In other words, a given dog has his normal protein level which shows only slight fluctuations, but this level may differ considerably from the level of another normal dog.

---

[1] Wm. J. Kerr, Sheldon Traveling Fellow in Research Medicine, 1916–1917, Harvard University, Cambridge, Massachusetts.

The earlier workers employed methods for the estimation of blood proteins which required large amounts of blood and at once introduced the factor of hemorrhage if many observations were to be recorded on the same animal within a short period of time. The labor necessary to isolate these various proteins was great and precluded any large series of observations by any single worker. We felt that it was very important to make frequent observations during any given experiment in order to get any clear understanding of the curve of blood protein regeneration uninfluenced by great loss of blood.

The refractometric method of Robertson (5) for the estimation of blood protein lends itself admirably to such work and made possible these experiments. It is of interest to note that our figures are in accord with those of Morawitz (6), who used the older methods of precipitation and analysis but very similar methods to deplete the blood proteins. This indicates that the figures obtained by the refractometer corresponded to those obtained by the other methods. We have recorded elsewhere (7) the values of blood proteins observed in normal dogs and a detailed description of the methods.

METHODS

Dogs were used for all these experiments and all remarks unless otherwise stated refer to dogs and their blood serum proteins. All operative procedures were done under complete surgical ether anaesthesia. So far as we are aware, Morawitz was the first worker to employ the method of *plasma depletion* as used in these experiments. The method is very simple and consists of bleeding the animal from the artery and introducing into the vein red corpuscles washed and suspended in saline solution, Locke's solution or some modification of a protein-free solution. The red cell Locke's solution mixture may be given during the arterial bleeding or after the bleeding. Morawitz used the former method. This method was used by Abel, Rowntree and Turner (8) and called "Plasmapharesis." We wish to use the term to indicate a withdrawal of whole blood and replacement of washed red corpuscles suspended in Locke's solution or some modification of this medium. However this procedure may be used it effects a dilution or depletion of the plasma and removes plasma proteins in large amounts. The experiment can be repeated frequently during the day and the blood proteins can be reduced to a low level without any anemia or fall in the volume of red blood cells. If these exchanges are done with care and proper regard for clinical evidences of shock, the plasma depletion can be carried from 6 per cent protein to 2 per cent and even as low as 1 per cent serum protein. If the exchanges are too rapid or too large in amount the dog will go into a condition of profound shock which will be discussed in detail in a future publication.

In our first experiments we had some trouble with subsequent infection at the site of operation in the neck or groin where the vessels were exposed, but improved technique did away with this factor and the wounds healed in two to three days with a minimum reaction. In these experiments the blood was drawn into clean glass containers into oxalate or citrate and centrifuged to separate the plasma. The red cells were washed at least twice to remove all proteins and suspended in Locke's solution or Locke's solution containing 3 to 4 per cent gum acacia made up to the original volume. These collections and washings were not done with complete aseptic precautions but some later experiments done with every aseptic caution gave identical results. In some experiments we used washed red corpuscles obtained from another normal dog, but in other experiments (tables 4, 5 and 6) the corpuscles were obtained from the same dog on the day before the complete experiment was carried out. No differences in experimental result can be attributed to these differences in red blood corpuscles.

The blood proteins were estimated by the refractometric method of Robertson (5). The greater part of the readings was made by the same individual and with but one exception the readings given in any one table were all made by the same individual to minimize fluctuations which may be due to personal factors.

Metabolism experiments were done in the usual cages which make possible an accurate collection of urine. The dogs were catheterized at the end of every 24-hour period, the bladder washed out, the animal weighed and given a uniform amount of water by stomach tube—usually 400 cc. The urinary collections and cage washings were made up to a unit volume and analyses of nitrogen by the Kjeldahl method done in duplicate.

The estimated blood volumes were calculated as 7.5 per cent of the body weight. Experiments soon to be published will indicate that this figure is below the actual blood volume figure which will average about 10 per cent according to the accurate determinations in the normal dog made possible by an improved dye method for blood volume estimation. The figures for total blood volume per cent exchange as given in the tables are actually about 30 per cent too high. We hope to report other experiments in which this factor is accurately controlled.

EXPERIMENTAL OBSERVATIONS

All the results are given in the form of tables and unless otherwise stated the dogs were in normal condition during the entire experiment. It will be noted that several series of observations (for example, tables 4 and 5) were made upon the same animal under the same or different experimental conditions. This gives additional data concerning the normal fluctuations of plasma proteins during varying intervals of time in the same animal. Table 1 is given here to furnish data of a characteristic experiment which shows the negative result of fasting and heavy feeding upon the blood serum proteins of a normal dog. This is in harmony with the work of other experimenters who used different methods. "Protein quotient" in table 1 is a figure obtained by dividing the albumin per cent by the globulin per cent and does not vary very greatly in a normal dog. With increase in the globulin this protein quotient approaches one and there may be inversion of the

TABLE 1

Serum albumin, globulin and total protein after feeding and after fasting

DATE	FEEDING OR FASTING	TOTAL PROTEIN	TOTAL ALBUMIN	TOTAL GLOBULIN	ALBUMIN	GLOBULIN	NON-PROTEIN CONSTITUENTS	PROTEIN QUOTIENT
					per cent	*per cent*	*per cent*	
December 30	Starved 3 days (?)	7.0	4.8	2.2	70	30	1.8	2.3
January 3	3 days of mixed diet 3 hours after meal	6.6	4.1	2.5	62	38	1.7	1.6
January 4	Bled 24 hours after feeding	6.4	4.5	1.9	70	30	1.6	2.3
January 10	6 days of fast	6.5	4.2	2.3	64	36	1.6	1.7
January 13	2 hours after feeding	6.1	4.0	2.1	65	35	1.6	1.9
January 27	After a feeding	5.4	3.6	1.8	66	34	2.0	1.9

normal ratio giving a protein quotient of 0.5 or even less. Such inversion is often present in infections and intoxications as pointed out in detail in other publications from this laboratory (7 and 9). The "protein quotient" is not figured in the other charts as we do not wish to emphasize this figure in these experiments.

Tables 2 and 3 give the results of two experiments which are good examples of others similarly performed. Table 2 illustrates a moderate depletion of serum proteins to only 60 per cent of normal. The body is able to regenerate to the normal serum protein level only after eight days, with zero food intake. It will be seen that in the days following the plasmapharesis the globulins tend to heap up faster than the albumins. This may be due to some infection during the experiment or more probably to the shock of the experiment as shown in later tables. It may be evidence that the serum globulins are regenerated

TABLE 2

Dog 16-4. Regeneration of serum proteins. Fasting

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total protein	Total albumin	Total globulin	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
May 4	5.5	4.0	1.5	2.2	22.50	Fasting begun
May 6	5.4	3.5	1.9	2.0		
May 10						
Specimen 1.	5.1	2.6	2.5	3.0	20.13	*Plasmapharesis:* Total blood withdrawn 505 cc. R. B. C. suspension injected 520 cc. done in 3 bleedings. Total proteins reduced 40 per cent
Specimen 2.	4.3	2.1	2.2	2.7		
Specimen 3.	4.2	2.4	1.8	2.4		
Specimen 4.	3.1	1.1	2.0	2.5		
May 11	3.6	1.9	1.7	2.5	19.13	Animal active. Wound in good condition
May 12	4.0	2.0	2.0	2.4	19.00	
May 13	3.1	1.5	1.6	2.4		
May 14	4.3	3.2	1.1	2.8	18.25	
May 15	4.3	2.0	2.3	2.6		
May 16	4.5	3.0	1.5	2.6	17.50	
May 17	3.8	1.0	2.8	2.6	17.13	
May 18	5.0	2.6	2.4	2.2	17.00	
May 19	5.0	2.5	2.5	2.0	16.00	
May 20	5.2	2.9	2.3	2.1	16.50	

faster than the albumins or that during this forced regeneration the precipitation values of these proteins may vary from normal.

Table 3 gives the results of a slightly different experiment. At the beginning of the experiment a large amount of Locke's solution was introduced and this was followed at once by the usual plasmapharesis

TABLE 3

Dog 16-113. Hydraemic plethora. Plasmapharesis. Regeneration of serum proteins. Fasting

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total protein	Total albumin	Total globulin	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
May 15.........	4.9	3.4	1.5	2.3	16.75	Bled 170 cc. into 20 cc. 10 per cent sodium citrate solution
May 16					16.25	Hydraemic plethora. 1000 cc. Locke's solution introduced into femoral vein before plasmapharesis
Specimen 1...	2.8	1.7	1.1	1.7		
Specimen 2...	2.5	1.6	0.9	2.0		
Specimen 3...	1.8	0.9	0.9	2.2		*Plasmapharesis:* Estimated blood volume 555 cc. Bled 1070 cc. in 4 bleedings of 150-400 cc. each. Total 192 per cent of blood volume. Reinjected 1920 cc.
Specimen 4...	1.6	0.8	0.8	2.3		
Specimen 5...	1.7	0.9	0.8	2.5		
May 17.........	2.5	1.2	1.3	2.1	16.25	Water ad lib.
May 18.........	2.9	1.3	1.6	2.4	15.75	Wound O.K.
May 19.........	3.2	1.8	1.4	2.3	15.50	
May 21.........	3.6	1.9	1.7	2.2	14.63	Slight hemolysis
May 22.........	3.7	1.7	2.0	2.0	14.25	
May 23.........	3.6	1.9	1.7	2.1	14.00	
May 24.........	3.9	1.9	2.0	1.9	13.63	
May 25.........	3.8	1.9	1.9	2.0	13.25	On bread and milk diet after bleeding
May 26.........	3.6	2.0	1.6	1.7	13.63	Bread and milk diet
May 28.........	3.9	2.1	1.8	1.8	13.25	Bread and milk diet
May 30.........	3.9	2.1	1.8	1.8	13.63	Meat diet after bleeding
May 31.........	4.0	1.6	2.4	1.9		Slight turbidity of serum. Meat diet
June 1..........	4.1	2.3	1.8	2.0	13.50	Meat diet
June 2..........	4.1	2.1	2.0	1.8	14.00	Meat diet
June 4..........	4.8	2.9	1.9	1.8	13.63	Experiment discontinued

during which 920 cc. of red blood cell suspension were injected and bleedings of 1070 cc. were removed. This procedure removes more blood protein but also intensifies the shock and the chances of a fatal result are greater. Removal of 192 per cent of the estimated blood volume lowered the blood proteins to 1.7 per cent The curve of regeneration is quite slow during nine days of fasting and was not rapid even on a meat diet. Thus table 3 illustrates the difficulty with which the body regenerates serum protein when the circulating protein is depleted and probably the reserve supply called out. During the first week there is a tendency for the globulins to increase more rapidly than the albumins.

The three tables below (tables 4, 5 and 6) are very similar and give interesting data concerning the basal nitrogen metabolism. Following the plasmapharesis there is a definite rise of nitrogen elimination in the urine—often one or two grams of nitrogen above the normal base line to be noted in each of the two days following the plasmapharesis. The curve of nitrogen elimination resembles exactly that reported by Whipple and Cook (10) following the intravenous injection of proteose material. We wish to assume that in both cases we are recording a *definite injury to body protein*, in one case due to the sudden dilution of the plasma and in the other case due to the action of toxic proteose on the body protein.

Furthermore, there is no evidence of any increased protein breakdown during the period of prolonged effort on the part of the body to build up the blood serum proteins. There is the period of two to three days or longer following the plasmapharesis in which the nitrogen elimination is much above normal; then follows a period of normal nitrogen elimination for a week or more while the body is still making up the serum protein deficit. During the last period there is no evidence that the body is breaking down protein to furnish the material to construct the necessary serum proteins. The protein breakdown is that normally observed in the fasting dog. Some observers may wish to explain the primary rise in urinary nitrogen after plasmapharesis as due to an emergency reaction to supply the urgently needed serum proteins. We believe the evidence is more in favor of the explanation that the rise in basal nitrogen elimination is due to actual injury of body protein associated with the shock of the plasma dilution. Table 6 gives an excellent example of acute and almost fatal shock following a small but rapid plasmapharesis. An exchange of 50 per cent of the estimated blood volume caused severe clinical shock with low blood pressure and temperature, vomiting and diarrhoea. Dog was in poor condition when removed to his cage. The recovery the next day was perfect and the rise in basal nitrogen is not marked.

TABLE 4

Regeneration of serum proteins. Metabolism

DATE	SERUM PROTEINS				NITROGEN ELIMINATION	WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents			
1917	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*pounds*	
February 19.....	5.2	3.6	1.6	2.0	1.68	19.38	400 cc. water daily by stomach tube
February 20.....	4.9	3.5	1.4	2.2	1.68	19.06	Bled 150 cc. into sodium citrate solution
February 21.....					2.10	18.44	*Plasmapharesis:* Estimated blood volume 630 cc. Bled 740 cc. in 5 bleedings of from 75 to 150 cc. each = 120 per cent of blood volume. Reinjected 845 cc. of Locke's acacia solution
Specimen 1....	4.7	3.5	1.2	2.4			
Specimen 2....	3.9	3.1	0.8	2.2			
Specimen 3....	3.1	1.8	1.3	2.4			
Specimen 4....	3.1	2.0	1.1	2.5			
Specimen 5....	2.6	2.0	0.6	2.9			
Specimen 6....	2.2	1.8	0.4	3.1			
February 22.....				3.1	3.89	18.44	Hemolysis
February 23.....	2.7	1.2	1.5	3.7	4.45	17.63	Hemolysis
February 24.....	3.1	1.1	2.0	3.3	3.14	17.13	400 cc. water by stomach tube
February 25.....					3.58	17.00	
February 26.....	3.5	2.0	1.5	2.9	3.00	16.63	
February 27.....	4.5	2.8	1.7	2.7	2.44	16.44	
February 28.....	4.1	2.3	1.8	2.5	2.13	16.19	
March 1.........	3.9	2.2	1.7	2.6	1.82	15.88	
March 2.........					1.74	15.56	75 gm. sugar in 400 cc. water by gavage
March 3.........	4.3	2.6	1.7	2.2	2.02	15.38	75 gm. sugar in 400 cc. water
March 4.........					1.57	14.88	
March 5.........	5.0	2.8	2.2	2.4	1.40	14.63	Small amount lean meat
March 6.........	4.9	3.1	1.8	2.5			Liberal meat diet
March 8.........	5.9	3.3	2.6	2.3		16.00	Liberal meat diet

Tables 4 and 5 give the results of similar experiments on the same dog at different times and illustrate beautifully the slow protein regeneration which follows the depletion of a plasmapharesis. The normal

TABLE 5

Dog 17-140. Regeneration of serum proteins. Metabolism

DATE	SERUM PROTEINS				NITROGEN ELIMINATION	WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents			
1917	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*pounds*	
April 24	4.9	3.5	1.4	2.4	2.41	20.25	Bled 220 cc. into citrate solution
April 25					2.38	19.19	*Plasmapharesis:* Estimated blood volume 690 cc. Bled 700 cc. in 4 bleedings of 150 to 180 cc. each. Total 101 per cent of blood volume. Reinjected 860 cc. in Locke's acacia solution
Specimen 1	4.5	3.0	1.5	2.1			
Specimen 2	4.3	3.1	1.2	2.0			
Specimen 3	3.7	2.6	1.1	2.1			
Specimen 4	3.4	2.5	0.9	2.2			
Specimen 5	2.3	1.7	0.6	2.8			
April 26	2.9	1.8	1.1	2.7	3.50	19.13	Slight infection of wound
April 27	3.2	1.7	1.5	2.8	3.30	18.25	400 cc. water daily by stomach tube
April 28	3.7	2.3	1.4	2.2		18.06	
April 29	3.0	1.3	1.7	1.3	2.38	17.88	Marked hemolysis
April 30	4.3	2.4	1.9	2.1	2.44	17.63	Moderate hemolysis
May 1	4.2	2.2	2.0	2.1	2.44	17.63	Moderate hemolysis
May 2					2.04	17.13	
May 3	4.2	1.8	2.4	2.3	2.38	16.81	
May 4	4.1	2.2	1.9	1.8	1.96	16.56	
May 5	4.0	1.9	2.1	2.0	1.93	16.31	
May 6					1.76	15.94	
May 7	4.1	2.4	1.7	1.8	1.99	15.75	Slight hemolysis
May 8	4.2	2.9	1.3	1.7	1.96	15.44	Moderate hemolysis
May 9	4.1	2.5	1.6	1.9	1.88	15.13	
May 10	4.6	2.7	1.9	1.8	2.07	15.00	Moderate hemolysis
May 11	4.7	2.7	2.0	1.9	2.04	14.81	Moderate hemolysis. 50 gm. cane sugar in water by gavage
May 12	4.4	2.4	2.0	2.0	1.62	14.25	50 gm. cane sugar and 25 gm. glucose in water by gavage
May 13	4.5	2.7	1.8	2.2			50 gm. cane sugar and 25 gm. glucose in water by gavage

TABLE 5—*Continued*

DATE	SERUM PROTEINS				NITROGEN ELIMINATION	WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents			
1917	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*pounds*	
May 14	4.4	2.7	1.7	2.4	1.48	14.13	50 gm. cane sugar and 25 gm. glucose in water by gavage
May 15	4.7	3.0	1.7	2.1	1.65	14.00	Metabolism discontinued. On bread and milk diet after bleeding
May 16	4.4	2.7	1.7	2.4		14.50	Bread and milk diet
May 17	4.3	2.6	1.7	2.1		15.25	Bread and milk diet
May 18	4.4	2.9	1.5	2.0		15.50	Bread and milk diet
May 19	4.3	3.1	1.2	2.0		15.88	Bread and milk diet
May 21	4.6	2.7	1.9	2.1		15.75	On meat diet after bleeding
May 22	5.0	3.1	1.9	2.2		15.50	Meat diet
May 23	5.0	3.2	1.8	2.2		16.06	Meat diet. Slight hemolysis
May 24	4.8	2.4	2.4	2.9		17.13	Moderate lipemia. Experiment discontinued

healthy fasting dog can regenerate serum proteins only with difficulty and complete repair from a depletion of 40 to 50 per cent takes many days—usually ten or more. In many similar experiments we have no record of complete regeneration to the normal level while the dog was fasting. When the depletion is not as great the regeneration may take the serum proteins practically back to normal in about ten days, with no food intake. Table 4 shows in an extreme degree the change which may appear in the non-protein fraction by this refraction metric method. During a plasmapharesis there is usually a rise in the non-protein fraction and this may appear on several days following the plasma depletion. The increase in this experiment is almost 50 per cent above normal (table 4) and the non-protein fraction remains high on the three succeeding days. There is no similar change in the non-protein nitrogen as determined by the usual methods—for example, by trochloracetic acid precipitation of serum proteins and Kjeldahl determinations of nitrogen in the filtrate. During a long plasmapharesis the urea and non-protein nitrogen of the blood plasma determined by

TABLE 6

Dog 17-150. Regeneration of serum proteins. Metabolism

DATE	SERUM PROTEINS				NITROGEN ELIMINATION	WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents			
1917	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*pounds*	
March 26	5.8	3.1	2.7	1.9	2.05	13.94	400 cc. water daily by stomach tube
March 27	5.6	3.1	2.5	1.7	2.13	13.75	Bled 150 cc. in 15 cc. 10 per cent sodium citrate solution
March 28					2.18	13.19	*Plasmaphoresis:* Estimated blood volume 450 cc. Bled 230 cc. in 2 bleedings = 50 per cent blood volume. Reinjected 450 cc. in Locke's acacia. Animal in shock. Diarrhoea. Returned to cage in poor condition
Specimen 1	5.3	3.0	2.3	1.5			
Specimen 2	4.4	2.6	1.8	1.7			
Specimen 3	2.9	1.2	1.7	2.1			
March 29	3.6	1.7	1.9	2.1	2.44	13.13	Good condition
March 30	3.9	2.0	1.9	2.2	2.63	12.63	400 cc. water daily by stomach tube
March 31	3.9	1.9	2.0	1.9	2.04	12.56	Wound O.K.
April 1					1.96	12.19	
April 2	4.2	2.1	2.1	1.4	1.88	12.19	
April 3	4.1	1.8	2.3	1.8	1.85	11.94	
April 4	4.2	1.7	2.5	1.9	1.74	11.81	
April 5	4.0	1.5	2.5	1.7	1.85	11.69	
April 6	3.6	0.9	2.7	1.8	1.97	11.19	
April 7	4.4	1.8	2.6	1.7	1.65	11.06	Slight hemolysis of serum
April 8					1.57	10.81	
April 9	4.4	1.4	3.0	2.0	1.60	10.88	Animal weak. Bread and milk diet. Small amount
April 10	4.6	2.6	2.0	2.0	1.51	10.50	Feedings increased
April 11	4.1	1.3	2.8	2.2		11.88	Extra diet
April 13	4.4	2.1	2.3	3.0		12.31	Lipaemic serum
April 14	4.9	2.2	2.7	2.4			Lipaemic serum
April 16	5.2	2.6	2.6	2.0		13.25	
April 17	4.9	2.4	2.5	1.9			
April 18	5.0	3.2	1.8	1.7			Experiment discontinued

any of the clinical methods will show a slight rise above normal—even at times 50 per cent above normal, but this change is very transient and returns to normal in a few hours.

The non-protein fraction as given in the charts represents a great variety of substances in the serum not precipitated by acetic acid—the salts, sugar, urea, etc. What substance explains the increase in the non-protein fraction under these conditions we do not venture to state. There may even be a change in the precipitation value of the proteins. Work is in progress which may throw some light on this point.

DISCUSSION

Many efforts have been made to show some definite relationship between the blood proteins, tissue proteins and food proteins. It is a natural enough assumption that the circulating blood proteins play an important part in nutrition of body cells, but all efforts to prove this point have failed and the more recent work on the amino acids makes it clear that plasma proteins do not necessarily represent a transition stage between food protein and body tissue protein. It is known that the blood proteins show no definite change in hunger or after heavy protein feeding, but we are familiar with other substances in the blood which maintain a very constant level in health (for example, glucose) where a rapid transfer is known to take place between the food and body tissues through the blood. So the constant level of blood proteins in hunger or after feeding gives no proof that the blood proteins are not concerned in the transfer of proteins from the food to the body tissues. But when by experiment we reduce the blood proteins to a low level (to 33 per cent or even to 20 per cent of normal) and follow the slow regeneration to normal in five to ten days on a meat diet we are fairly certain that these blood serum proteins cannot be actively concerned as *transition products* of protein metabolism standing between food proteins and body tissue proteins; otherwise the return to normal would be expected to take place within twenty-four hours or less. We are convinced that these experiments give sufficient proof that the blood serum proteins are not directly concerned in the protein metabolism as intermediary products of exchange between food protein and body tissue protein. We hope to submit evidence to show that these blood serum proteins serve a very important function in maintaining the normal equilibrium of the parenchyma cells.

When the serum proteins have been depleted to a low level by means of repeated plasmapharesis the dog will show a slow regeneration of these plasma proteins. On starvation this curve may not rise to normal in ten to fourteen days yet the nitrogen elimination in the urine may be normal after the first few days. There will be no variation from normal in the blood urea and non-protein nitrogen after the evidences of clinical shock have vanished. Yet we cannot say with conviction that the body does or does not break down any excess body protein to form the serum proteins which surely are formed during this period of zero protein intake. We may assume that the body conserves certain amino acids and builds them into the necessary serum proteins but there is no evidence of nitrogen retention (tables 4, 5 and 6). It must be admitted that the body may break down cell protein and recast it into serum protein without changing the basal nitrogen elimination. These several possibilities are to be considered and at this time we cannot emphasize one to the exclusion of another.

SUMMARY

Serum proteins may be reduced to a low level in the normal dog by means of repeated bleedings and simultaneous injections of washed blood cells suspended in some protein-free medium (e.g., Locke's solution).

Regeneration of the serum proteins may be followed after this type of plasma depletion (plasmapharesis). This regeneration is a slow and difficult matter occupying many days—often seven to fourteen days when the original depletion removed 50 per cent or more of the total serum protein.

This depletion presumably is the maximal stimulus for body production of serum protein, yet there is a marked delay in regeneration. It seems that the body can produce serum proteins only in small amounts even in an emergency.

Serum proteins can be regenerated when there is no protein intake in the food, therefore the serum proteins must be formed from the body proteins under such circumstances.

The basal nitrogen metabolism shows no constant variation under these experimental conditions, with the exception of a primary rise in nitrogen elimination which occurs in the few days following the shock of the plasmapharesis.

There is a tendency for the curve of globulin regeneration to rise faster than that of the serum albumin. This may be due to the initial shock of the plasmapharesis or to some infection and slight wound reaction. It may possibly be due to more rapid production of serum globulin.

We have no evidence of any constant fluctuation in serum proteins in association with periods of fasting or heavy protein feeding. The changes observed come within the limits observed in normal dogs under uniform conditions.

These experiments give no evidence that the serum proteins may be in any way concerned as intermediary products between food protein and body tissue or parenchyma protein.

BIBLIOGRAPHY

(1) Whipple: This Journal, 1914, xxxiii, 50.
(2) Goodpasture: This Journal, 1914, xxxiii, 70.
(3) Morawitz: Oppenheimer's Handb. d. Biochem. d. Menschen v. d. Tiere, 1909, ii, 70.
(4) Rowe: Arch. Int. Med., 1916, xviii, 455.
(5) Robertson: Journ. Biol. Chem., 1915, xxii, 233.
(6) Morawitz: Beitr. z. Chem. Phys. u. Path., 1906, vii, 153
(7) Hurwitz and Whipple: Journ. Exper. Med., 1917, xxv, 231.
(8) Abel, Rowntree and Turner: Journ. Pharm. Exper. Therap., 1914, v, 625.
(9) Hurwitz and Meyer: Journ. Exper. Med., 1916, xxiv, 515.
(10) Whipple and Cooke: Journ. Exper. Med., 1917, xxv, 461.

REGENERATION OF BLOOD SERUM PROTEIN

II. Influence of Diet upon Curve of Protein Regeneration Following Plasma Depletion

WM. J. KERR,[1] S. H. HURWITZ and G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication October 21, 1918

The experiments tabulated below supplement those in the preceding paper and must be compared with them in any analysis of results. The experiments were performed under identical conditions described previously and the figures may be compared without any allowance being necessary to cover individual variations in technique. The conditions of the experiments and methods have been described in the preceding communication.

It is evident that the curve of serum protein regeneration rises more promptly to normal when the dog is on a full diet as compared with a similar experiment upon a fasting animal. A meat diet seems to be most efficient in effecting a rapid regeneration of serum protein. Even under the most favorable conditions of diet it requires from five to eight days to regenerate the serum proteins to normal when the depletion has been carried to 50 per cent or 33 per cent of normal. This shows that the body cannot form serum protein with much more speed than it can form new cells in parenchymatous organs (e.g., liver cells).

EXPERIMENTAL OBSERVATIONS

Tables 10 and 11 show very clearly the sharp rise in the regeneration curve of serum proteins which is associated with a *meat diet*. In the first experiment the depletion of serum protein was not extreme — a reduction to 2.9 per cent — and the regeneration may be considered complete in five days, although the abnormally high figure of the first reading is not attained. There was no notable increase in the globulin fraction.

---

[1] Wm. J. Kerr, Sheldon Traveling Fellow in Research Medicine, 1916–1917, Harvard University, Cambridge, Massachusetts.

The second experiment shows a great depletion of the serum proteins effected by eight bleedings amounting to 157 per cent of the estimated blood volume. The rise in the non-protein fraction is well illustrated. The curve of regeneration is rapid and complete in eight days in spite of the initial low level.

TABLE 10

Dog 16–129. Regeneration of serum proteins. Meat diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
March 15						
Specimen 1...	6.4	5.0	1.4	2.2	14.00	*Plasmapharesis:* Estimated blood volume 480 cc. Four bleedings each of 135 cc. Total withdrawn 540 cc. Reinjected 600 cc. Total protein reduced 55 per cent
Specimen 2...	5.4	4.1	1.3	2.1		
Specimen 3...	4.7	4.0	0.7	2.2		
Specimen 4...	3.5	2.5	1.0	2.4		
Specimen 5...	2.9	1.8	1.1	2.7	13.75	
March 16.......	3.6	2.3	1.3	2.0	14.00	Dog active. Wound in good condition
March 17.......	4.2	2.5	1.7	1.9	13.50	Meat diet
March 18.......	4.4	2.8	1.6	1.8	14.50	
March 19.......	4.5	2.1	2.4	2.0	15.00	
March 20.......	5.0	3.0	2.0	1.9		
March 22.......	5.1	3.0	2.1	2.2	15.50	
March 29.......	5.4	3.8	1.6	2.2	17.00	

Tables 12 and 13 show the curve of serum protein regeneration on a "mixed diet." By this term we mean a diet of table scraps including bones, meat, bread scraps, potatoes, rice, etc., given in liberal amounts once per day. Such a diet of course is rich in a variety of proteins. Table 12 records the serum protein fluctuations in an experiment in which the plasma depletion was considerable and the shock very severe. Two exchanges were performed each of 500 cc. which was slightly more than the total estimated blood volume. Such large exchanges are always associated with shock and often with fatal shock.

This dog showed profound shock and had not recovered the next day when blood-stained feces were passed. The depletion of the blood plasma proteins is not as great as attained by a series of smaller bleedings but the figures are relatively low. In spite of the profound shock, the regeneration of total proteins during the first twenty-four hours amounts to a little more than 1 per cent. The regeneration may be looked upon as complete in six days. There was practically no upset in the globulin-albumin ratio in spite of the shock and slight infection of the neck wound.

TABLE 11

Dog 13-140. Regeneration of serum proteins. Meat diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
February 26	4.9	3.2	1.7	3.1	23.25	
February 27	4.6	2.6	2.0	2.8		Bled 175 cc. into 20 cc. 10 per cent sodium citrate solution
February 28					23.00	*Plasmapharesis:* Estimated blood volume 787 cc. Bled 1250 cc Seven bleedings from 140 to 200 cc. each. Total 157 per cent of blood volume. Reinjected 1325 cc. in Locke-acacia solution
Specimen 1.	4.8	3.2	1.6	2.6		
Specimen 2.	4.1	2.8	1.3	2.2		
Specimen 3.	2.6	1.8	0.8	3.3		
Specimen 4.	3.4	2.5	0.9	2.5		
Specimen 5.	2.5	1.8	0.7	3.3		
Specimen 6.	2.2	1.8	0.4	3.5		
Specimen 7.	1.8	1.3	0.5	3.6		
Specimen 8.	1.7	1.3	0.4	3.6		
March 1	2.5	1.2	1.3	3.3	22.25	Dog normal
March 2	3.2	1.5	1.7	3.2	22.50	
March 3	4.0	2.3	1.7	2.5		
March 5	4.2	2.3	1.9	2.1		
March 6	4.4	2.4	2.0	2.0	23.00	
March 8	5.0	2.6	2.4	2.0	22.50	

Table 13 shows an experiment in which the depletion was effected by smaller exchanges but a lower final figure was attained than in the preceding experiment (table 12). The non-protein fraction shows the rise usually noted. There was a minimum amount of shock and the dog was in excellent condition at the end of the experiment. In spite of the great difference in the general condition of the two dogs (tables 12 and 13) the amount of serum protein regeneration during the first twenty-four hours following the plasmapharesis is identical. It will be noted that this figure is constant in most of the experiments regardless of shock or its absence or abundant food or fasting. It seems that this must be an emergency reaction and represents the supreme effort on the part of the body.

TABLE 12

Dog 16–106. Regeneration of serum proteins. Mixed diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
February 16						*Plasmapharesis:* Estimated blood volume 472 cc. Two bleedings, each of 500 cc. R. B. C. for first injection obtained on day previous from another animal and suspended in Locke-acacia solution
Specimen 1	6.0	5.4	0.6	2.0	14.00	
Specimen 2	3.1	1.3	1.8	2.8		
Specimen 3	2.0	1.5	0.5	3.0		
February 17	3.2	2.0	1.2	2.5	13.75	Dog drowsy—passed some bloody urine and bloody stool. Does not eat. Temperature 38.7°
February 18	3.5	2.9	0.6	2.8	14.25	Dog more active. Eating. Some suppuration of neck wound
February 19	4.4	3.6	0.8	2.1		Mixed diet—dog normal
February 20	4.5	2.9	1.6	2.2		
February 21	4.9	2.0	2.9	1.9	14.50	
February 22	5.5	3.8	1.7	1.8		
February 23	4.8	2.6	2.2	1.8		
February 24	5.4	3.3	2.1	1.5	15.00	
February 26	5.1	3.4	1.7	2.0		

Table 14 shows an experiment which is complicated by infection and suppuration. The globulins show a considerable rise at the expense of the albumins. These fluctuations occur while the curve of total protein regeneration follows a slow upward trend. There seems to be more delay in this experiment than usual in uncomplicated experiments with mixed diet. We have other observations to indicate that a severe infection will tend to slow up the speed of regeneration. It is not certain whether this delay may not be explained at least in part by impaired appetite and digestion. The primary depletion was to an extremely low level as can be seen by comparison with other experiments. We find that 1 per cent is about the lowest figure obtainable

TABLE 13

Dog 17-69. Regeneration of serum proteins. Mixed diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
January 29, 30.	5.3	2.9	2.4	2.4	15.00	Bled 75 cc. into 7.5 cc. 10 per cent sodium citrate solution
January 31					15.00	*Plasmapharesis:* Estimated blood volume 510 cc. Five bleedings from 85 to 150 cc. each. Total 710 cc. Reinjected 885 cc. in Locke-acacia solution. Bleedings totaled 144 per cent of blood volume.
Specimen 1...	3.2	1.2	2.0	2.8		
Specimen 2...	2.7	1.8	0.9	3.3		
Specimen 3..	2.3	1.8	0.5	3.4		
Specimen 4...	1.8	1.0	0.8	3.4		
Specimen 5...	1.4	0.4	1.0	3.6	15.50	Returned to cage in good condition
February 1 .	2.5	1.2	1.3	3.2	15.50	Animal active. Mixed diet
February 2 ...	3.7	1.2	2.5	2.5		Wound in neck slightly septic
February 3 ..	3.6	1.7	1.9	2.9		Neck septic
February 4 ..		2.1		3.8		Lipemic serum
February 5 ...	3.7	2.4	1.3	2.7		Wound healing
February 6....	4.4	1.8	2.6	2.4		
February 7 ..	4.1	2.6	1.5	2.4		Wound O.K.
February 8 ..	5.0	3.5	1.5	2.2	14.50	
February 12 ..	5.1	4.4	0.7	2.4	14.25	Experiment discontinued

by plasma depletion. It seems that 1 per cent serum protein is an absolute minimum below which the body cells cannot survive. Many dogs will show fatal shock before the serum proteins are reduced even to 2 per cent and there is much individual variation in resistance to the shock of plasmapharesis. This dog required twelve days to regenerate approximately to normal. The complicating amputation was due to a thrombus probably the result of infection and trauma. The animal was sacrificed at the end of the experiment and autopsy revealed no abnormalities except those described.

Table 15 shows an uncomplicated experiment with serum protein regeneration following a moderate depletion, the dog being on a liberal bread and milk diet. There was no severe shock and no infection, yet we note on several days a definite increase of globulins at the expense of the albumins. The curve of total protein regeneration is uniform but the speed of regeneration is not as great as upon a meat diet. This table also gives figures to indicate a moderate rise in the blood non-protein nitrogen during the various stages of the plasma depletion. Such changes are much more marked when there is severe

TABLE 14

Dog 16–127. Regeneration of serum proteins. Mixed diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
March 8........						
Specimen 1...	6.4	6.0	0.4	2.1	15.50	*Plasmapharesis:* Estimated blood volume 525 cc. Five bleedings 135 cc. each. Total amount of blood removed 675 cc. Reinjected 750 cc. Total protein reduced 83 per cent
Specimen 2...	3.9	3.2	0.7	2.4		
Specimen 3...	2.9	2.4	0.5	3.0		
Specimen 4...	2.3	2.1	0.2	3.4		
Specimen 5...	1.4	1.1	0.3	4.1		
Specimen 6...	1.1	1.1	0	4.3	15.88	
March 9.......	2.4	1.5	0.9	3.0		Animal drowsy; oedema of left foreleg
March 10.......	2.6	1.3	1.3	2.8		Eats little
March 11.......	3.0	1.3	1.7	3.0	15.00	
March 12.......	3.3	1.6	1.7	2.5		Dog ill. Temperature 40.0°. Moist gangrene of left foreleg
March 13.......	3.8	2.6	1.2	2.0	14.50	Amputation of left foreleg
March 14.......	3.3	1.0	2.3	2.1		
March 15.......	3.8	1.5	2.3	2.0	13.50	Dog active. Eats well
March 16.......	3.6	0.6	3.0	2.0	14.00	Abscess on back
March 17.......	4.2	2.4	1.8	1.8		Abscess incised
March 20.......	5.4	1.5	3.9	1.8	14.00	Dog active. Eats. Abscess wound granulating

clinical shock which is associated with considerable breakdown of body protein (see preceding paper).

TABLE 15

Dog 16-124. Regeneration of serum proteins. Bread and milk diet

DATE	SERUM PROTEIN				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globuline	Non-protein constituents		
	per cent	*per cent*	*per cent*	*per cent*	*pounds*	
1916						
March 22.......						
Specimen 1...	6.1	3.6	2.5	2.7	16.00	*Plasmapharesis:* Total withdrawn 600 cc. Reinjected 600 cc.
Specimen 2...	4.0	2.7	1.3	2.8		Blood N. P. Nitrogen 37 mgm., urea 28 mgm.
Specimen 3...	3.1	1.6	1.5	2.9		Blood N. P. N. 38 mgm., urea 28 mgm.
Specimen 4...	2.6	2.2	0.4	3.0		Blood N. P. N. 46 mgm., urea 27 mgm.
March 23	3.7	2.5	1.2	2.5	16.00	Dog active. Wound in good condition
March 24	3.9	2.6	1.3	2.2		Bread and milk 400 grams
March 25	4.5	2.6	1.9	1.8	16.00	Bread and milk 100 grams
March 27	4.6	2.0	2.6	2.0	16.00	Bread and milk 500 grams
March 28	4.5	1.6	2.9	2.2	15.75	Very active
March 29	4.9	1.7	3.2	1.9	16.00	Bread and milk 500 grams
April 1	5.0	2.1	2.9	2.1	15.50	Bread and milk 450 grams
April 3	5.2	3.0	2.2	2.1	16.00	

DISCUSSION

It is of interest to recall the fact that the body regenerates its *cell protoplasm* with the same difficulty and delay following cell injury. This statement applies particularly to the parenchymatous organs, as the liver and kidney. Whipple and Sperry (1) have shown that the liver injured by chloroform will repair the damaged cell protoplasm within seven to ten days, depending upon the severity of the original cell necrosis. Mr. Davis in this laboratory is investigating the curve of the liver cell regeneration and finds certain points of similarity between the parenchyma regeneration and the blood serum protein regeneration. It may well be that the protein for the parenchyma cell or for the blood plasma may require similar construction periods and building material It may be that the blood protein construction may depend upon the activity of the cell protoplasm. It is possible that all protein material may be built up part way alike, to be differentiated in the last stage of the complete elaboration.

We wish to point out the fact that after the initial depletion of serum proteins the body can almost always regenerate 1 per cent total protein during the first twenty-four hours following the plasmapharesis. This figure is remarkably constant and does not seem to be influenced by diet or fasting. The amount of shock does not appear to influence it because it may be the same in two experiments one of which is associated with grave shock (table 12) and the other with a minimum amount of shock (table 13). We may perhaps look upon this as the maximum effort of the body to replace these essential serum proteins, an effort which surely depends upon the body protein as it is present in fasting experiments. It will be the same whether there is a marked breakdown of host protein as evidenced by great increase in urinary nitrogen or whether this tissue autolysis is minimal.

SUMMARY

Regeneration of serum proteins is more rapid and complete upon a meat or mixed diet as compared with fasting experiments see (fig. 1).

The same regeneration does not appear to be as rapid upon a bread and milk diet as upon a meat diet.

Following the plasma depletion there is an average regeneration of 1 per cent total serum proteins within the next twenty-four hours. This is a uniform emergency reaction on the part of the body and is independent of food, shock or infection. It probably represents the absolute maximum production under the greatest stimulus and perhaps represents a throwing in of emergency reserve material.

The body has the same difficulty in regenerating its serum proteins, as it does in the construction of cell protein—for example, the repair of liver protoplasm following a chloroform necrosis.

Regeneration of serum protein after a 50 per cent depletion on a meat diet may be effected in five to seven days.

Regeneration of liver cell protoplasm after a 50 per cent necrosis may be effected in five to seven days. This is an interesting parallel which may have some significance.

The body cannot tolerate a reduction of its total serum protein from an average of 5 per cent to the low level of 1 per cent. This is practically always associated with fatal shock which may supervene long before the minimum figure of 1 per cent has been attained.

BIBLIOGRAPHY

(1) Whipple and Sperry: Johns Hopkins Hosp. Bull., 1909, xx, 278.

REGENERATION OF BLOOD SERUM PROTEINS

III. LIVER INJURY ALONE: LIVER INJURY AND PLASMA DEPLETION: THE ECK FISTULA COMBINED WITH PLASMA DEPLETION

WM. J. KERR,[1] S. H. HURWITZ AND G. H. WHIPPLE

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication October 21, 1918

The two preceding communications make it clear that the body can regenerate its serum proteins more rapidly and completely upon a full diet than during a fasting period. At best this reaction is sluggish even under the most intense stimulus and requires several days (see fig. 1).

We wish to submit experiments to show that liver injury tends to lessen the amount of circulating blood serum proteins and the presence of liver injury tends to retard the regeneration of serum proteins after an initial plasma depletion. Furthermore the Eck fistula liver appears to regenerate blood serum proteins more slowly than the normal or even the injured liver. The Eck fistula liver is smaller than normal, always presents evidence of degeneration (fat) and gives a subnormal functional test with tetrachlorphthalein (1).

It is to be recalled that the widest fluctuation in fibrinogen, which is an important plasma protein, will occur in parallel relationship to liver injury produced by chloroform or phosphorus (2). It is very easy to cause a wide dissociation between the curves of fibrinogen and of serum proteins. For example, a fatal chloroform poisoning will reduce the fibrinogen from 0.5 per cent to almost zero, but this may cause only a moderate fall in serum proteins of 2 per cent, perhaps from the normal level of 5 per cent to 3 per cent the day before death.

The fibrinogen is a very labile substance which seems to react to abnormal conditions with great speed and can be produced in an emergency in large amounts; for example, the fibrinogen can be completely washed out of the blood but it will promptly return in a few hours (3, 4). The importance of this reaction in relation to hemorrhage is obvious as the fibrinogen forms the tough body of all blood clots.

---

[1] Wm. J. Kerr, Sheldon Traveling Fellow in Research Medicine, 1916–1917, Harvard University, Cambridge, Massachusetts.

We have seen that the serum proteins are more stable substances which cannot be regenerated in any short period of time. These proteins cannot be removed in any large amount without causing fatal shock, yet the serum proteins have some features in common with the plasma protein fibrinogen. There is some evidence in this paper to indicate an important relationship between the serum proteins and the liver, just as has been established in the case of fibrinogen. It is not too much to assume that the liver may be concerned in the production of serum protein and the maintenance of these vitally important fluid proteins at a normal level in the blood.

It is possible to make a graphic comparison of the curves of serum protein regeneration as influenced by diets, starvation and liver injury (see fig. 1). The curve of liver injury is the average of three experiments similar to table 23 with the use of chloroform or phosphorus to cause liver necrosis.

The methods have all been described in the first papers of this series. Experimental conditions were identical with those previously described.

EXPERIMENTAL OBSERVATIONS

Tables 20, 21 and 22 may be considered as a group. The normal level of the serum proteins is somewhat influenced by a fatal phosphorus poisoning, perhaps more than appears at first sight. Table 20 shows an experiment in which a normal dog was given about one-half a lethal dose of phosphorus dissolved in oil subcutaneously. The dog gave no clinical reaction but there was a slight fall in the curve of serum proteins. One week later the dog was given a lethal dose of phosphorus which was fatal in three days. There was a considerable fall in the blood serum proteins but we must not forget that this fall is probably greater than the figures indicate. Dogs poisoned with phosphorus vomit a good deal, show concentrated urine, have a tendency to hemorrhage and show a blood hematocrit indicating a relative increase in red cells. All this points to a decrease in blood volume at the expense of the plasma fraction and this means a fall in the *total serum proteins* of the body, more than is indicated by an analysis of a unit specimen of serum.

The liver of fatal phosphorus poisoning needs no detailed description. It is large and obviously fatty, specked with ecchymoses. The microscope shows many necrotic liver cells but especially a marked fatty degeneration of the cell protoplasm in every liver lobule.

Table 21 shows the result of a lethal dose of phosphorus which killed the dog in five days. There is a progressive fall in total serum proteins with the advance of the clinical symptoms of intoxication. The fibrinogen in both dogs was practically absent in the blood on the day before death. This explains the general hemorrhagic tendency and purpura. The blood obtained post-mortem gives a high protein reading but this has no significance because so many factors cannot be

TABLE 20

Dog 17-126. Phosphorus poisoning. Serum proteins. Mixed diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
March 10	5.0	2.8	2.2	1.7	12.88	
March 12	4.9	2.5	2.4	1.8	12.88	Given subcutaneously ¾ mgm. phosphorus in oil per pound body weight
March 13	4.5	2.2	2.3	1.7	12.75	
March 14	5.0	2.9	2.1	1.7	12.25	
March 15	4.8	3.0	1.8	1.8		
March 16	4.9	2.9	2.0	1.9		
March 17	4.7	2.5	2.2	1.9	12.50	No evidence of phosphorus poisoning
March 19	4.9	2.9	2.0	1.6	11.88	Given subcutaneously 1¼ mgm. phosphorus in oil per pound body weight
March 21	4.9	2.9	2.0	1.9	12.00	Serum bile-stained. Fibrinolytic test faintly positive. Not eating. Vomiting
March 22	3.8	2.0	1.8	1.6	11.75	Distinctly sick. Not eating. Vomiting. Plasma wine-colored. Clot does not form after several hours. Animal gradually failed and died in p.m. Liver necrosis and fat with multiple hemorrhages

determined. There is usually much blood concentration under such conditions and loss of fluid to the tissues.

Table 22 gives the results of a mild chloroform poisoning which influences only slightly the level of blood serum proteins. The data of this table are to be compared with those in the two following tables where the chloroform injury is combined with a plasma depletion. It will be noted (table 22) that the fall in total serum proteins begins on the second day after the chloroform anaesthesia and continues during the next three or four days during which period we know that the liver repair is most active. It can be estimated from all data at hand

TABLE 21

Dog 17-129. Phosphorus poisoning. Serum proteins. Mixed diet

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
March 16	6.2	4.5	1.7	2.3	11.50	Given 1¼ mgm. phosphorus in oil subcutaneously per pound body weight
March 17	5.7	3.4	2.3	2.2	12.00	
March 18	4.8	2.5	2.3	2.1		Not eating. Vomiting. Serum bile-tinged
March 19	4.4	2.3	2.1	2.2	11.13	Not eating. Vomiting. Purpuric spots on neck. Serum deeply bile-tinged. Coagulation time delayed.
March 20	3.8	0.6(?)	3.2	3.9	10.75	Vomiting. Not eating. Purpura increasing. Difficult to bleed. Serum deeply bile-stained. Serum diluted for readings. Coagulation time delayed
March 21	5.8	3.4	2.4	2.6		Death during night. Multiple hemorrhages. Liver necrosis Specimen taken from heart's blood for readings. Plasma mahogany-red color. Does not clot
						Serum post-mortem

that this chloroform anaesthesia for one and one-half hour did not cause more than one-third necrosis of each liver lobule. Furthermore, we know that such injuries can be repaired to normal in five to seven days. It will be observed that the dog was in excellent condition and gained weight during this period of repair. The notes on the diet were lost but it is practically certain that the dog was on a mixed diet.

Tables 23 and 24 are to be compared as the first shows the effects of a mild chloroform poisoning and the second of a more severe chloroform poisoning combined with a plasma depletion. Table 23 also is to be compared with table 6 in the first paper of this series which shows the result of a preceding plasma depletion done upon this same dog with metabolism figures. This preceding experiment was associated with severe shock and the curve of nitrogen metabolism with and without the chloroform necrosis is very similar. We may assume that the amount of liver injury (1 hour anaesthesia) in table 23 was very slight or that the severe shock of table 6 caused as much disturbance in the animal as did the chloroform poisoning—the increase in nitrogen elimination being about the same. The influence in any case is not

TABLE 22

Dog 16–9. Chloroform poisoning. Serum proteins

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
May 1	6.1	4.5	1.6	2.0		Mixed diet (?)
May 2	5.5	3.2	2.3	2.1		Chloroform anaesthesia 1½ hours. Well taken
May 3	5.9	4.3	1.6	2.4		
May 4	5.0	2.6	2.4	2.1	38.25	
May 5	4.7	2.7	2.0	2.3		
May 6	4.6	2.7	1.9	2.2		
May 7	4.4	1.8	2.6	2.6		
May 8	4.9	3.3	2.6	2.7		
May 9	4.9	2.6	2.3	2.8	40.13	
May 11	5.6	2.8	2.8	2.2		
May 13	5.3	2.9	2.4	2.7		
May 17	5.5	2.6	2.9	2.1		Complete recovery

very evident in the curve of serum protein regeneration. When the dog is placed on a meat diet the curve rises promptly to normal.

TABLE 23

Dog 17-130. Plasma depletion. Chloroform necrosis. Regeneration of serum proteins

DATE	SERUM PROTEINS				NITROGEN ELIMINATION	WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents			
1917	*per cent*	*per cent*	*per cent*	*per cent*	*grams*	*pounds*	
May 1..........	4.5	2.8	1.7	2.3	1.32	13.81	Bled 80 cc. into 8 cc. of 10 per cent citrate solution
May 2					1.18	13.06	*Plasmapharesis:* Estimated blood volume 465 cc. Four bleedings from 70 to 150 cc. each Total 505 cc. = 109 per cent of blood volume. Reinjected 700 cc. in Locke-acacia solution
Specimen 1....	5.2	3.3	1.9	2.1			
Specimen 2....	3.9	2.3	1.6	2.1			
Specimen 3....	3.4	1.9	1.5	2.4			
Specimen 4....	2.6	1.3	1.3	2.6			
Specimen 5....	2.0*	1.4	0.6*	2.7			
May	3.1	1.1	2.0	2.6	1.99	12.81	Chloroform necrosis. -1 hour light 10 cc. used
May 4......	3.0	1.6	1.4	2.3	2.80	12.44	Slight hemolysis of serum
May 5......	3.2	1.3	1.9	2.9	3.08	11.88	Serum slighly bile-stained
May 6..........					1.76	11.69	400 cc. water each day by stomach tube
May 7.........	3.4	1.3	2.1	2.4	1.79	11.56	
May 8.........	3.8	2.0	1.8	2.1	1.62	11.38	
May 9.........	4.2	2.2	2.0	2.3	1.46	11.13	
May 10.........					1.54	10.94	
May 11.........	4.3	2.1	2.2	2.1	1.51	10.75	
May 12	4.7	2.1	2.6	2.4	1.60	10.44	Meat diet after bleeding
May 13.........	4.9	2.2	2.7	2.5		11.50	Lipemic serum
May 14....... .	4.7	2.6	2.1	2.5			
May 15	5.2	2.7	2.5	2.8		11.63	Serum slightly turbid
May 16	5.7	3.0	2.7	2.7			Serum slightly turbid
May 17	5.3	2.8	2.5	2.5		12.13	Serum slightly turbid

Table 24 brings out several points of interest. The plasma depletion was to a moderately low level but not associated with severe shock. More chloroform was given to this dog than to the dog in the preceding experiment (table 23) and the presence of coloring matter in the serum for four days indicates a considerable amount of liver injury. The dog was clinically disturbed on the day after the anaesthesia and refused food, which indicates considerable intoxication. There is a loss of weight in spite of the fact that the dog had access to food in the cage. The amount of food eaten is not known but the dog had

TABLE 24

Dog 17-186. Plasma depletion. Chloroform necrosis. Regeneration of serum proteins

DATE	SERUM PROTEIN				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
April 17	5.7	3.4	2.3	1.7	21.00	Bled 200 cc. into 20 cc. 10 per cent sodium citrate solution
April 18					20.88	*Plasmapharesis*: Estimated blood volume 713 cc. in 4 bleedings from 130 to 200 cc. each or 98 per cent of blood volume. Reinjected 910 cc.
Specimen 1	5.5	3.4	2.1	2.0		
Specimen 2	4.2	2.5	1.7	2.0		
Specimen 3	3.9	2.9	1.0	2.2		
Specimen 4	3.3	2.0	1.3	2.4		
Specimen 5	2.1	0.9	1.2	2.8		
April 19	3.0	1.9	1.1	3.1		Light chloroform anaesthesia for 1 hour. 15 cc. chloroform used. Serum normal color
April 20	2.9	1.3	1.6	2.8	20.88	Animal sick. Drinks only small amount of milk. Serum canary-yellow color (bile)
April 21	3.0	1.4	1.6	3.0	19.75	Serum slightly bile-tinged
April 22	3.0	0.9	2.1	2.6		Serum slightly bile-tinged
April 23	4.0	2.2	1.8	2.3	19.00	Serum slightly tinged
April 24	2.9	0.8	2.1	3.1		Serum normal color
April 25	4.2	2.4	1.8	2.6	19.75	Serum normal color
April 26	3.6	1.0	2.6	2.9	19.00	
April 27	4.2	2.4	1.8	3.1	18.25	Animal quiet. Discharge from eyes and nose. Distemper
April 28	3.4	2.0	1.4	3.9	18.13	Animal distinctly sick. Distemper. Serum clear
April 29	3.0	0.8	2.2	3.4		
April 30						Found dead. Broncho-pneumonia. Liver necrosis

bread and milk in the cage and evidently ate some of it as can be estimated from the weight curve.

The curve of serum proteins shows a slow rise toward normal followed by a terminal fall during the two days before death which was due to distemper. Autopsy showed lungs involved with bronchopneumonia, edema and congestion. Liver shows necrosis in the center of each lobule. The central three-fifths of each liver lobule is very fatty and full of large vacuolated liver cells, some fresh fibrin and leucocytes of all types. The marginal two-fifths of the liver lobule is more nearly normal. The repair of this liver necrosis is much delayed and abortive. Ten days elapsed since the chloroform injury and liver necrosis of this amount should be completely repaired within five to eight days under usual conditions. The great delay in liver repair was due in part to the intoxication and loss of appetite as well as to the intercurrent infection (distemper). The reaction on the curve of blood serum regeneration is not very profound when we consider the injury done the liver, the small food intake and the fatal outcome.

Table 25 is to be compared with the two preceding tables where the injury is due to chloroform. The type of reaction is similar and the reaction to the liver injury of phosphorus is quite definite. We are fortunate in being able to refer to other experiments upon this same dog (tables 4 and 5 in the first paper of this series) where the curve of regeneration has been influenced by fasting. The influence of phosphorus poisoning is a little more marked than the long period of fasting. During the phosphorus experiment the dog was on a liberal mixed diet but lost some weight and showed a few symptoms of intoxication. This dog received about two-thirds of a lethal dose of phosphorus. Also refer to another experiment upon this same dog (table 11). The regeneration curve of serum proteins is influenced by a meat diet and this experiment gives a perfect control to table 25.

Tables 26 and 27 are to be considered together as the experiments are similar and the results practically identical. The Eck fistula operations were performed some time before the plasma depletion as indicated in each table. The operation consists in an anastomosis between the portal vein and the vena cava by means of special clamps and a ligation of the portal vein above the anastomosis.

Table 26 gives the figures of a very successful experiment in which the plasma depletion was carried out with a minimum amount of shock. Following this exchange the dog was in excellent condition and maintained the usual weight curves. It will be seen that there is scarcely any regeneration of serum proteins during the three days following the plasmapharesis, in spite of a liberal diet and a relatively slight plasma depletion. In a normal dog under similar conditions the regeneration would be practically complete (compare table 10 in the preceding paper). A bile fistula was performed on October 7 and the dog died on November 16 from typical Eck fistula intoxication. Au-

TABLE 25

Dog 16-140. Plasma depletion. Regeneration of serum proteins. Phosphorus poisoning

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1917	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
April 2.........	5.2	3.2	2.0	2.0	25.00	Mixed diet
April 3.........	5.7	3.7	2.0	2.0	25.00	Bled 180 cc. into 20 cc. 10 per cent sodium citrate solution
April 4					25.00	*Plasmapharesis:* Estimated blood volume 855 cc. Bled 970 cc. in 5 bleedings of from 100 to 210 cc. Total 113 per cent of blood volume. Reinjected 1100 cc. Non-protein nitrogen (Kjeldahl) 28.1 mgm. per 100 cc. blood
Specimen 1...	5.4	3.2	2.2	1.9		
Specimen 2...	4.3	2.2	2.1	1.7		
Specimen 3...	3.3	1.9	1.4	2.3		
Specimen 4...	3.2	1.6	1.6	2.2		
Specimen 5...	2.8	1.6	1.2	2.4		
Specimen 6...	1.9	0.3	1.6	2.7		
April 5.........	2.8	1.3	1.5	3.0	23.50	At 11.00 a.m. given ¾ mgm. phosphorus per pound body weight subcutaneously in oil
April 6.........	2.5	0.5	2.0	3.8	22.50	Slight vomiting
April 7.........	3.2	1.2	2.0	3.1	22.25	Leg wound doing well
April 9.........	4.0	2.2	1.8	2.4	21.50	No evidence of phosphorus poisoning
April 10.........	3.8	1.5	2.3	2.3	21.00	Mixed diet
April 11.........	3.6	1.2	2.4	2.8	21.50	
April 13.........	4.5	2.2	2.3	2.2	21.75	
April 14.........	4.2	1.6	2.6	2.8	22.25	Cloudy serum (lipemia)
April 16.........	4.3	1.6	2.7	2.4	23.00	
April 17.........	4.6	2.3	2.3	1.9	23.25	
April 18.........	4.9	2.9	2.0	2.0	23.38	
April 19.........	5.0	2.9	2.1	2.1	23.00	Serum slightly turbid
April 20.........	4.9	2.9	2.0	2.0	23.25	Experiment discontinued

topsy showed that the Eck fistula was widely patent but the ligature on the portal vein was not completely occluding, a tiny opening persisting at this place. Some portal blood could gain access to the liver but it must have been small in amount and did not prevent the dog from developing the typical Eck fistula intoxication.

TABLE 26

Dog 16-115. Eck fistula. Plasma depletion. Regeneration of serum proteins

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
April 5........					18.25	Eck fistula operation: February 22. *Plasmapharesis:* Total blood withdrawn 675 cc.; total injected, 700 cc. Total proteins reduced 44 per cent. Estimated exchange = 108 per cent of blood volume
Specimen 1.	5.5	3.9	1.6	1.7		
Specimen 2.	4.4	3.3	1.1	1.7		
Specimen 3..	3.6	2.2	1.4	1.8		
Specimen 4.	3.1	1.7	1.4	1.7	17.88	
April 6........	3.6	2.4	0.2	1.6	17.50	Animal in good condition. Wound O.K.
April 7.......	3.5	2.1	1.4	1.9	18.00	Animal active. Bread and milk diet
April 8.........	3.4	1.3	2.1	1.7		
April 9.......	3.7	1.7	2.0	1.7		
April 10.......	3.9	2.4	1.9	1.7	18.13	
April 11.......	4.2	2.4	1.8	1.7	18.00	
April 12........	3.8	2.1	1.7	1.7		
April 13.......	4.0	2.0	2.0	1.9	18.50	
April 14........	3.8	2.1	1.7	1.9		
April 15.........	3.5	1.2	2.3	2.3		
April 17.......	3.4	1.5	1.9	2.1		
April 20.......	4.0	2.2	1.8	2.3	18.75	
April 24........	4.8	3.7	1.1	2.2	19.00	
May 1.......	4.8	2.4	2.4	1.4	19.00	

Table 27 gives striking results and shows a complete absence of serum protein regeneration during the twenty-four hours after the experiment. The initial shock of the plasmapharesis was considerable in spite of the fact that the plasma exchange amounted to only 59 per cent of the estimated blood volume. The initial blood serum readings were low and the regeneration of serum protein very slow. The dog was on a liberal diet and lost no weight. On June 28 the dog developed the typical Eck fistula intoxication. Autopsy showed a patent Eck fistula and the ligature on the portal vein completely occluding it. The liver showed the usual atrophy and central fatty degeneration which is always present in the Eck fistula dog.

Figure 1 gives a graphic comparison of curves which represent the blood serum regeneration under different conditions. Curve *D* is the average of seven experiments showing the serum protein regeneration on a meat diet or a full mixed diet. Curve *S* represents the average of five experiment with fasting periods after the plasma depletion. Curve *O* represents the average of three experiments in which the liver was injured on the day following the plasma depletion using chloroform or phosphorus. This curve is less regular because there are fewer experiments which in the average tend to smooth out minor fluctuations. This chart brings out the constant regeneration of about 1 per cent

TABLE 27

Dog 16–93. Eck fistula and splenectomy. Plasma depletion. Regeneration of serum proteins

DATE	SERUM PROTEINS				WEIGHT	REMARKS
	Total proteins	Total albumins	Total globulins	Non-protein constituents		
1916	*per cent*	*per cent*	*per cent*	*per cent*	*pounds*	
April 19.........						Eck fistula operation March 10. Splenectomy March 23. *Plasmapharesis:* Total blood withdrawn 600 cc. R. B. C. suspension injected 600 cc. Estimated exchange = 73 per cent of blood volume
Specimen 1...	4.6	2.2	2.4	2.3	24.00	
Specimen 2...	4.1	1.2	2.9	3.0		
Specimen 3...	4.1	1.5	2.6	2.2		
Specimen 4...	3.4	2.1	1.3	2.6		
April 20.........	3.1	1.2	1.9	2.1	23.25	Animal very weak. 32 gm. glucose by stomach tube
April 21.........	3.7	2.3	1.4	1.7	23.25	Bread and milk diet
April 22.........	3.6	1.1	2.5	2.0	22.25	
April 24.........	3.8	2.5	1.3	2.5		
April 25.........	3.8	2.5	1.3	2.2	23.13	
April 26.........	4.1	1.6	2.5	1.7		
May 2.........	4.4	1.2	3.2	1.9	23.63	

total protein in the twenty-four hours following the experimental procedure of plasma depletion. In spite of food intake the curve of serum protein regeneration following liver injury is less steep than the fasting curve.

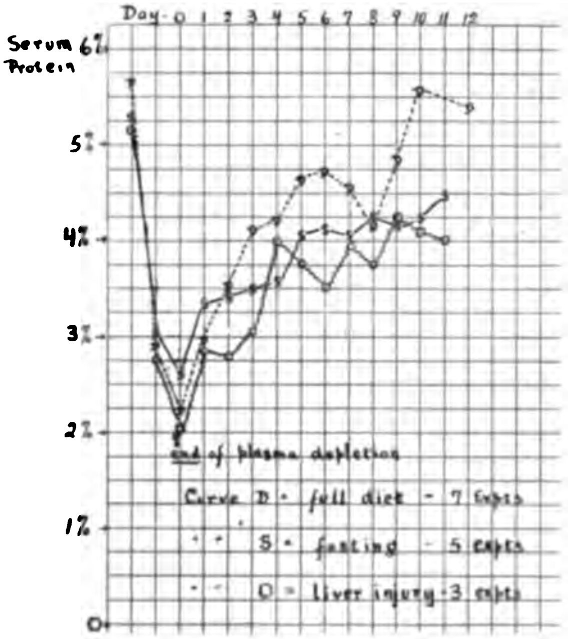


Fig. 1.

DISCUSSION

We do not wish to lose sight of the fact that the curve of blood serum regeneration is not profoundly influenced by a coincident liver necrosis (table 24) and a part of the slow regeneration may be attributed to the *low food intake* which results from the liver injury and consequent intoxication. But the amount of reserve power of the liver must not be disregarded in this complicated reaction. On the other hand we should not forget the fact that the serum proteins fall as a result of fatal liver injury.

A very important point too is that the Eck fistula liver is associated with a peculiar curve of blood serum regeneration. It will be recalled (fig. 1) that practically every experiment in plasma depletion shows a rise of about 1 per cent during the twenty-four hours following the plasma removal. This indicates perhaps an emergency production or an emergency reserve which helps bring the serum proteins back to a safe level. The Eck fistula dog does not show this 1 per cent rise after the plasma depletion and shows a slow serum protein regeneration in spite of a generous diet. The plasma depletion can never be carried to a very low level because these dogs are very susceptible to the shock of this procedure. There is clearly much difficulty in serum protein regeneration by the Eck fistula dog. The liver of the Eck fistula dog is known to be insufficient in several capacities. These Eck fistula dogs die from peculiar intoxications associated with convulsions and in spite of considerable experimental work, the correct explanation of this intoxication has not yet been advanced.

Much experimental evidence points to the liver as being concerned in the maintenance of a normal level of blood serum proteins. The evidence here is not as convincing and striking as is the case in the plasma protein *fibrinogen* which has such an intimate relationship to liver injury and disappears almost completely with acute liver injury. This all tends to emphasize the *stability* of the blood serum protein even under unusual conditions. We rarely find much fluctuation in the blood serum protein level in varying conditions of health or disease.

SUMMARY

Pronounced injury of the liver by means of phosphorus or chloroform will cause, or at least be associated with, a moderate fall in blood serum proteins.

Regeneration of blood serum proteins following a plasma depletion is slow at best but will be delayed by the presence of liver injury due to phosphorus or chloroform.

The Eck fistula liver is smaller than normal and is functionally deficient in some respects. Eck fistula dogs show a distinct inability to regenerate blood serum proteins following a plasma depletion. The rapid regeneration of the first twenty-four hours is especially lacking and these dogs are very susceptible to the shock of the plasma dilution.

All this experimental evidence points to the liver as concerned in maintaining the normal level of the blood serum proteins.

There is a *remarkable stability of the blood serum protein level* in widely varying conditions of health and disease.

BIBLIOGRAPHY

(1) **Whipple, Peightal and Clark**: Johns Hopkins Hosp. Bull., 1913, xxiv, 343.
(2) **Whipple and Hurwitz**: Journ. Exper. Med., 1911, xiii, 136.
(3) **Whipple**: This Journal, 1914, xxxiii, 50.
(4) **Goodpasture**: This Journal, 1914, xxxiii, 70.

THE NORMAL GROWTH OF WHITE LEGHORN CHICKENS

G. DAVIS BUCKNER, R. H. WILKINS AND JOSEPH H. KASTLE[1]

From the Chemistry Department of the Kentucky Agricultural Experiment Station, Lexington

Received for publication October 22, 1918

The White Leghorn chick has been used by a number of scientists for nutritional experiments with varying degrees of success. That the White Leghorn chicken lends itself most usefully to experimentation is seen by the fact that, being a very highly nervous animal, it reacts quickly to any change of nourishment or stimulation. A value also exists in that the Leghorn breed grows rapidly, reaching maturity in less than a year. There are certain physical symptoms which are readily recognized as being caused by malnutrition or some pathological condition. Principal among these visible symptoms are the appearance of the skin on the abdomen, the condition of the vent and the anemic and drooping condition of the comb and wattles, which show only a rudimentary development if the chick does not receive the proper food during the period of growth. Each of the above named conditions would, in all probability, point to some disturbance in the respiratory or circulatory systems or to faulty diet. The drooping of the wings might mean chronic or acute digestive disorders, in the latter case the wing-drooping disappearing in a few hours. For these and other reasons the White Leghorn chick has been of great service in this laboratory, fulfilling the desired conditions of experiments now in progress.

In view of this fact it seemed most important that we definitely establish the normal curve of growth of this breed of chickens, so that an accurate control could be made of our laboratory experiments. It would naturally follow that any results obtained in the laboratory which showed a different rate of growth would be called abnormal and deductions would be made accordingly.

---

[1] The authors desire to express their appreciation to Prof. J. J. Hooper of the Animal Husbandry Department of this Station for generously allowing this investigation to be made at the poultry farm.

In order to cover all conditions controlling our experiments we used two lots of pure-bred White Leghorn chickens which had come from the same parent stock, each lot composed of sixty one-day-old chicks.

Lot 1 was raised under artificial conditions as follows: Two hundred eggs were hatched in an incubator and from the chicks obtained sixty were selected entirely at random and placed in a brooder-house which opened into a grass space containing 240 square feet of well-sodded earth. These chicks received numbered leg-bands and were weighed individually the day following hatching and were so weighed every week thereafter, on a torsion balance sensitive to 0.1 gram, individual records being kept. When a chick died from any cause its previous weight-record was discarded so that, at the end of twenty-eight weeks, we compiled our results from the individual records of the chicks remaining at that time. This was done in order to avoid any error in the average weight of the chicks caused by a possible loss in weight previous to death. The ration used was the standard Cornell ration which is as follows, the chemical analysis being of the particular grain mixtures fed.

Lot 2 was raised under natural conditions as follows: Two hundred White Leghorn chicks from the same parent stock as lot 1 were hatched under hens and when the chicks were one day old, sixty were selected at random and placed under four mother hens, being individually weighed at that time and weekly weighings and records made as of lot 1. The chicks were cared for by the mother hens and allowed to roam at will during the day over a large grass range and were housed at night in an ordinary coop. No special care was given them other than seeing that they were properly fed and watered. At the age of 6 weeks they were weaned from the mother hens and were placed in a pen containing an appropriate coop and with access to one-acre ranges. At this time lot 1 was removed to a pen having conditions in every way similar to those of lot 2 the feed for lot 2 being the same as that of lot 1. As soon as treading began the cockerels and pullets were separated to avoid any undue exercise on the part of the pullets, which might cause loss of weight. The mortality records of these two lots of chicks were kept to determine the variation in the vitality which might be stimulated by the freedom and natural care afforded by the mother hen during early growth.

	ANALYSIS	USED AS FOLLOWS
	Per cent	
Mixture 1 8 parts rolled oats 8 parts bread crumbs 2 parts sifted beef-scrap 1 part bone meal	Protein = 23.15 Ether extract = 5.30	1 to 5 days. No. 1, moistened with sour skimmed milk, fed five times each day. Mixture 2 in shallow tray containing a little of No.3 (dry) always before chicks, tray containing grit and charcoal. Sprouted oats once each day
Mixture 2 3 parts cracked wheat 2 parts cracked corn 1 part pinhead oatmeal	Protein = 12.30 Ether extract = 4.50	5 days to 2 weeks. No. 2 in litter twice a day. No. 3, moistened with sour skimmed milk, fed three times a day. No. 3 (dry) always available
Mixture 3 2 parts wheat bran 3 parts corn meal 3 parts wheat middlings 3 parts beef scrap (best grade) 1 part bone meal	Protein = 27.30 Ether extract = 6.20	2 to 3 weeks. As shown above except that the moist mash is given twice a day
Mixture 4 3 parts wheat (whole) 3 parts cracked corn 1 part hulled oats	Protein = 12.20 Ether extract = 4.10	4 to 6 weeks. Moist mash once a day; mixture 4 in litter twice a day; dry mash always available
Mixture 5 3 parts wheat 3 parts cracked corn	Protein = 11.30 Ether extract = 2.80	6 weeks to maturity. Nos. 3 and 5 hopper-fed. One meal a day of moist mash

Fine grit, charcoal, oyster shell and water were provided from the start.

The following table gives the average weekly weights of the chicks that remained at the close of this experiment and also the weekly mortality records of the entire lots.

TABLE 1

Average weights and mortality records of White Leghorn chicks

DATE	NUMBER OF WEEKS	LOT 1				LOT 2			
		Average weight of 1 chicken			Number surviving each week	Average weight of 1 chicken			Number surviving each week
		Cockerels	Pullets	Mean		Cockerels	Pullets	Mean	
April 18		42.1	41.1	41.6	60	42.2	40.9	41.6	60
April 25	1	72.2	69.2	70.7	55	76.8	75.2	76.0	54
May 2	2	109.7	95.6	102.6	53	108.2	101.3	104.7	54
May 9	3	161.4	140.9	151.1	53	151.2	145.0	148.1	54
May 16	4	231.6	191.7	211.6	53	209.5	198.3	203.9	54
May 23	5	292.2	248.8	270.5	53	283.8	262.4	273.1	53
May 30	6	360.9	288.3	324.6	52	374.2	328.0	351.1	53
June 6	7	442.8	354.4	398.6	52	437.8	383.6	412.2	53
June 13	8	496.9	395.6	446.2	52	580.6	490.7	535.6	52
June 20	9	537.6	409.9	473.7	51	616.6	552.8	584.4	51
June 27	10	630.9	488.7	559.8	48	729.2	648.9	689.0	51
July 4	11	674.4	506.4	590.4	48	758.4	655.5	706.9	50
July 11	12	812.9	622.2	717.5	48	926.9	775.4	851.1	50
July 18	13	902.3	682.4	792.3	48	992.7	826.8	909.7	50
July 25	14	953.2	695.1	824.1	48	1050.0	869.7	959.8	50
August 1	15	980.4	717.3	848.8	48	1106.8	898.0	1002.4	50
August 8	16	1063.2	780.9	922.1	48	1167.3	938.3	1052.8	50
August 15	17	1154.9	841.8	986.6	48	1235.6	987.2	1111.4	50
August 22	18	1252.7	889.4	1071.0	48	1282.7	1037.2	1160.0	50
August 29	19	1295.3	922.9	1109.1	47	1323.0	1045.3	1181.1	50
September 5	20	1344.8	956.2	1150.5	47	1365.4	1110.7	1238.0	50
September 12	21	1396.7	992.2	1194.4	47	1417.5	1149.2	1283.3	50
September 19	22	1430.6	1038.4	1234.5	47	1459.4	1233.1	1346.2	50
September 26	23	1442.6	1047.6	1245.1	47	1493.2	1249.4	1371.3	50
October 3	24	1462.1	1066.2	1264.1	47	1585.6	1329.9	1457.7	50
October 10	25	1480.0	1082.6	1281.3	45	1590.1	1339.0	1464.5	50
October 17	26	1528.5	1075.4	1301.9	43	1649.1	1368.7	1508.9	50
October 24	27	1560.6	1090.2	1325.4	43	1673.4	1405.4	1539.4	50
October 31	28	1594.6	1120.4	1357.5	42	1748.1	1447.5	1597.8	50

From the foregoing table and the graphs it will be seen that the average weight of one-day-old White Leghorn chicks is 41.6 grams and also that the average cockerel is 1.15 gram or 2.8 per cent heavier than the female of similar age. The superiority in the weight of the cockerels continues throughout the growing period. Further, it is seen that at the end of twenty-eight weeks the average chick, allowed the freedom and care of a mother hen, develops a vitality which manifests itself in allowing those chicks to become 240.3 grams heavier than those raised under artificial conditions as described. This is also borne out by the mortality figures. Each lot started with sixty chicks and at the end of twenty-eight weeks ten had died from some unknown and unavoidable causes in the hen-raised lot as against eighteen deaths in the artificially raised lot.

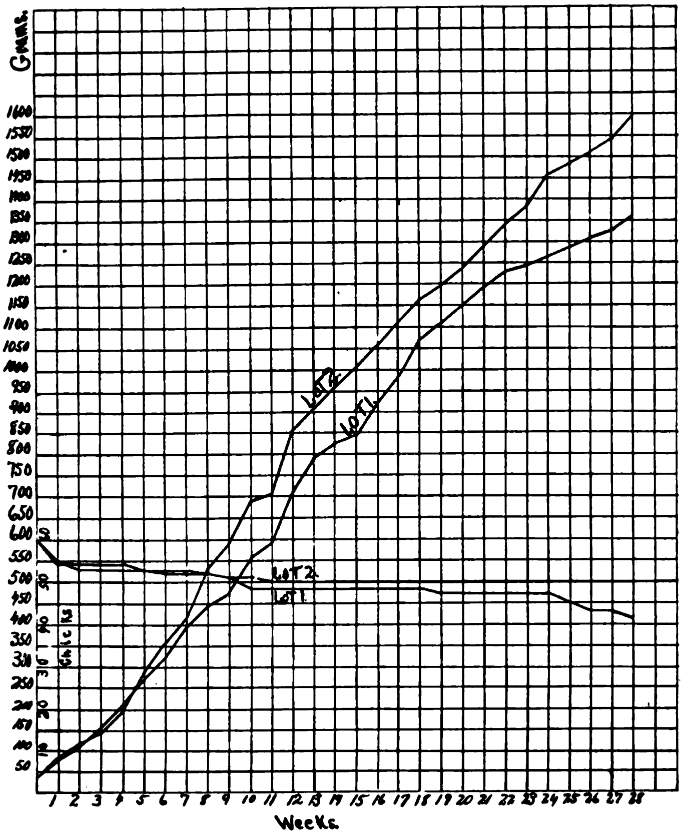


Fig. 1. Chart showing the normal rate of growth and mortality records of brooder raised (lot 1) and hen raised (lot 2) chicks.

Finally, from our results we feel justified in drawing the following conclusions:

The rate of growth of White Leghorn chicks, as shown by the graph and tabulation presented herein, closely approximates the normal.

The vitality of hen-raised chicks is superior to that of artificially raised chicks, as shown by weight and mortality.

That physical conditions governing the first few weeks of growth markedly affect the vitality of the chick, which is manifest during the entire period of growth.

SECRETIN

IV. THE NUMBER OF RED AND WHITE CORPUSCLES IN THE CIRCULATING BLOOD DURING DIGESTION

ARDREY W. DOWNS AND NATHAN B. EDDY

From the Physiological Laboratory of McGill University, Montreal, Canada

Received for publication October 24, 1918

In a previous report (1) we have shown that in the rabbit the subcutaneous injection of secretin brings about a considerable increase in the number of red and white corpuscles in the blood stream by directly stimulating the production of new cells. Bayliss and Starling (2) in their original investigation of the pancreatic mechanism demonstrated the presence of secretin in the intestine of the rabbit as well as in that of man, the cat, dog and other animals, and concluded that it is an identical substance in all the vertebrata. According to the hypothesis of these authors and the actual demonstration of Wertheimer (3) secretin is carried in the blood during digestion and should then be at least as potent a stimulus to blood cell formation as when experimentally introduced into the circulation. The examination of the peripheral blood during digestion, therefore, ought to reveal a change in the corpuscle content comparable with our experimental findings, i.e., an increase in the number of erythrocytes and leucocytes per unit volume.

The first work in this connection appears to have been done by Moleschott (4) in 1854. He found an increase in the proportion of white corpuscles to erythrocytes which was particularly marked if the diet was rich in protein food. Hofmeister (5) in 1887 called attention to the "cellular richness" of the adenoid tissue of the intestinal wall during digestion on a meat diet and Pohl (6) went on to show that an increase in the number of white corpuscles in the circulating blood appears early in digestion and persists for several hours. Goodall, Gulland and Paton (7) and Gulland and Paton (8) went into this subject in greatest detail and found in dogs that the maximum leucocyte count occurs three to four hours after a meal. They also showed this to be chiefly due to a very constant increase in the number of lymphocytes, contributed to much less regularly by a relative increase in the polymorphonuclear cells. They further demonstrated that the source of the leucocytosis is entirely, or almost entirely, the bone marrow in which they found an increased rate of production of white corpuscles during digestion.

Much fewer are the reported observations upon the erythrocytes during digestion. Pohl (6) found a slight diminution in their number, but he made very few determinations and did not follow the count through the entire period of digestion in any case. Buntzen and Sörensen (9) reported an increase of 8 to 25 per cent and Andressen (10) a decrease of 5 to 12 per cent in the number of erythrocytes per unit volume of blood in digestion. In estimations made before and after feeding Goodall, Gulland and Paton (7) found no change in the percentage of haemoglobin.

Källmark (11) in fasting experiments on rabbits found a slight falling off in the total leucocyte count during the fast and, as the most marked change in the differential count, a relative diminution of the lymphocytes. When the animals were again fed the total leucocyte count and the relative proportion of lymphocytes both increased above the original level. If the rabbits were allowed plenty of water he noted no change in the number of erythrocytes. According to Cabot (12) "food calls forth a greater leucocytosis in proportion as it is a novelty to the stomach." Rogers (13) and Swirski (14) in their work on the hunger contractions of the rabbit's stomach found that, even if the rabbits were put in a wire-bottomed cage, when all food was withheld they would eat their excreta and their stomachs would remain continuously partly filled; but if the animal's head was suitably fastened, the stomach completely emptied itself in about twenty-four hours. Therefore, the slight changes observed by Källmark may possibly be explained by his apparent failure to insure a total fast in his rabbits in some such manner.

We have been unable to find any record of blood counts during digestion in rabbits which have been made to endure a total fast. In view of this fact it seemed worth while to undertake such an investigation of the red and white corpuscles.

For this purpose apparently normal rabbits were selected and placed in a contrivance which might be aptly described as a pillory. This consisted of a box partitioned into two compartments. The partition was in two halves, an upper and a lower, in each of which was a rounded opening. These together made an ovoid aperture through which passed the neck of the rabbit. When in use the rabbit's head projected on one side of the partition while his body was on the other side. With the animal thus fixed an absolute fast was readily maintained. Each rabbit was kept in this box forty to forty-four hours without food but was given as much water as it would drink.

At the end of the fasting period both the red and white corpuscles were counted. The animal was then released and put into a cage where it had free access to food and water. The food in every case consisted of green cabbage and oats. The rabbit always began eating at once and ate more or less continuously all day. Erythrocyte and leucocyte counts were made hourly for the six hours following the beginning of the feeding. As in our previous work the blood was obtained from the ear of the rabbit. The diluting fluids used were 0.5 per cent acetic acid for the white corpuscles and physiological saline solution for the red corpuscles. The counts were made in the usual manner with the Thoma-Zeiss apparatus.

We have made in all ten determinations, the results of which are summarized in tables 1 and 2. These show an average increase of 76.78 per cent in the white corpuscles and of 18.65 per cent in the red corpuscles. The maximum leucocyte count occurred 3.2 hours, and the maximum erythrocyte count 1.9 hours after the feeding started.

To what extent are these changes in the corpuscle content of the blood during digestion in the rabbit comparable with the effect of the experimental administration of secretin? The subcutaneous injection of 1 cc. of secretin solution (10 mgm. of a dried acid extract) per kilogram of body weight is capable of causing in the rabbit an increase in the red corpuscles per unit volume of blood of 17.07 per cent, appearing in 50 minutes and persisting 63.33 minutes. The same dose will bring about an increase in the white corpuscles per unit volume of 44.2 per cent, appearing in 51 minutes and persisting 78.5 minutes (15). This experimental effect of secretin is less, appears more quickly and persists for a shorter time than the findings in digestion noted above. Assuming that secretin is responsible for the latter one would expect such differences. Our experimental solution must necessarily be less active than the secretin entering the blood during digestion; a certain delay must ensue after the feeding starts before food begins to leave the stomach and secretin to be formed; also the formation of secretin and its entrance into the circulation is presumably continuous though not uniform throughout digestion once the process is started.

As mentioned above Gulland and Paton (8) determined that the source of the digestion leucocytosis is an increased rate of production of white corpuscles by the bone marrow. We have demonstrated (1) that secretin acts by directly stimulating the production of both red and white corpuscles by the bone marrow. May it not be that the increased activity of the bone marrow in digestion is the result of the direct stimulating action of secretin?

TABLE 1

Red corpuscles during digestion in the rabbit

ANIMAL	COUNT BEFORE FEEDING	1ST HOUR	2D HOUR	3D HOUR	4TH HOUR	5TH HOUR	6TH HOUR	PERCENTAGE INCREASE	MAXIMUM IN
									hrs.
1	5,920,000	5,788,000	6,504,000	5,456,000	5,920,000	5,888,000	5,408,000	9.86	2
2	6,584,000	7,360,000	7,712,000	6,920,000	6,320,000	6,064,000	6,368,000	17.13	2
3	5,968,000	5,624,000	5,464,000	6,800,000	7,136,000	4,960,000	5,352,000	19.56	4
4	5,756,000	7,040,000	6,144,000	6,544,000	6,208,000	6,960,000	6,378,000	22.30	1
5	5,488,000	6,320,000	6,880,000	5,896,000	6,008,000	5,600,000	6,192,000	25.36	2
1	5,536,000	6,736,000	5,784,000	4,976,000	6,176,000	5,886,000	5,320,000	21.67	1
2	6,032,000	7,328,000	6,288,000	5,792,000	6,304,000	5,360,000	5,304,000	21.48	1
6	5,736,000	6,128,000	6,512,000	4,800,000	5,600,000	6,092,000	5,720,000	13.52	2
7	6,240,000	6,560,000	6,880,000	6,592,000	5,936,000	5,864,000	5,896,000	10.25	2
8	6,200,000	6,960,000	7,776,000	7,008,000	5,876,000	5,916,000	6,164,000	25.41	2

TABLE 2

White corpuscles during digestion in the rabbit

ANIMAL	COUNT BEFORE FEEDING	1ST HOUR	2D HOUR	3D HOUR	4TH HOUR	5TH HOUR	6TH HOUR	PERCENTAGE INCREASE	MAXIMUM IN
									hours
1	8,000	22,000	16,600	16,000	20,000	16,000	12,000	175.00	1
2	10,000	15,000	13,000	12,700	16,000	10,000	6,600	60.00	4
3	14,000	15,000	24,000	18,600	13,000	21,800	15,000	71.42	2
4	15,000	18,000	19,000	22,400	20,000	20,700	12,600	49.33	3
5	23,400	24,000	28,000	18,000	25,000	20,000	17,600	19.65	2
1	11,000	12,000	15,000	16,000	14,200	19,400	14,000	76.36	5
2	9,600	11,500	13,000	17,500	16,800	17,000	9,000	82.29	3
6	16,000	14,000	22,100	19,000	35,000	18,400	14,000	118.74	4
7	13,200	12,500	21,600	18,000	22,000	14,000	12,000	66.66	4
8	12,400	13,200	16,000	17,000	18,400	16,400	14,500	48.38	4

Additional evidence of the change in the corpuscle content of the blood during digestion being due to secretin is afforded by the differential leucocyte count. The findings of Goodall, Gulland and Paton (7) and of Källmark (11) indicate that, while the change in the leucocyte count during digestion is participated in by all the varieties of white cells, the greatest increase occurs in the lymphocytes. We have previously reported (16) the differential leucocyte count after the administration of secretin as compared to the normal; there also, while all forms are increased in number, the lymphocytes show the greatest relative increase.

CONCLUSIONS

1. During digestion there occurs an increase in the number of both the red and white corpuscles per unit volume of blood.
2. These changes are comparable with the effects produced by the experimental administration of secretin.
3. The source of the increased corpuscle content of the blood both in digestion and after the administration of secretin is an increased rate of production of new cells by the bone marrow.
4. The similarity between the differential leucocyte counts in digestion and after the administration of secretin is additional evidence of secretin being the cause of the increase in the number of corpuscles during digestion.

BIBLIOGRAPHY

(1) Downs and Eddy: This Journal, 1918, xlvi, 209.
(2) Bayliss and Starling: Journ. Physiol., 1903, xxix, 174.
(3) Wertheimer: Journ. d. Physiol. et Path. gén., 1902, iv, 1069.
(4) Moleschott: Quoted by Pohl, Arch. f. exper. Path. u. Pharm., 1889, xxv, 32.
(5) Hofmeister: Arch. f. exper. Path. u. Pharm., 1887, xxii, 306.
(6) Pohl: Arch. f. exper. Path. u. Pharm., 1889, xxv, 31.
(7) Goodall, Gulland and Paton: Journ. Physiol., 1904, xxx, 1.
(8) Gulland and Paton: Journ. Physiol., 1905, xxxiii, 20.
(9) Buntzen and Sörensen: Hoffmann-Schwalbe's Jahresb., 1879, xii, 232.
(10) Andreessen: Hoffmann-Schwalbe's Jahresb., 1883, xviii, 61.
(11) Källmark: Folia Haematologica, 1911, xi, 411.
(12) Cabot: Clinical examination of the blood, 1900, 84.
(13) Rogers: This Journal, 1915, xxxvi, 183.
(14) Swirski: Arch. f. exper. Path. u. Path.-Anat., 1898, xli, 143.
(15) Downs and Eddy: This Journal, 1918, xlv, 295.
(16) Downs and Eddy: This Journal, 1918, xlvi, 217.

POSSIBLE PERIODIC VARIATIONS IN THE EXTENT OF THE KNEE-JERK IN WOMEN

JESSIE L. KING

From the Department of Physiology, Goucher College, Baltimore

Received for publication October 28, 1918

The numerous attempts made to determine by physiological and psychological tests whether there is a monthly cycle in the life-processes of women have failed to give definite positive results, except as concerns the slight constant variations in pulse and temperature observed by many workers (1). Recent metabolism experiments add further evidence against the old theory of a rhythmical movement for they show that there is no pronounced periodic variation in the metabolism of either nitrogen, phosphorus or calcium (2), (3), (4).

The rise in temperature immediately preceding and accompanying the onset of menstruation may account for the feeling of discomfort—a certain amount of irritability and mental depression—which is frequently felt by normal women not troubled by dysmenorrhea. Yet it seems possible that there may be some variation in the tone of the nerve centers.

With the hope of throwing light on the question, at Doctor Howell's suggestion, I made a series of observations on a group of young women, using the knee-jerk as the physiological index of tonus. It is well recognized that the extent of the knee-jerk depends on the activity of the nerve centers, increasing or diminishing by whatever increases or diminishes the tone of the central nervous system (5), (6), (7), (8). Further the reflex is easily and constantly elicited from practically all normal individuals. An experiment in which amplitude alone is employed as an index of variation suffers under certain disadvantages for, as Lombard, Bodwitch and Warren and others have shown, although the amplitude is in general proportional to the blow, various interesting and obscure conditions may cause remarkable variations (7), (9). Dodge (6) warns especially against using amplitude "as an indicator of the variations of any one selected factor." It was considered, however, that averages of a large number of readings taken over periods of several weeks might be at least suggestive of general tendencies.

METHOD AND OBSERVATION

The apparatus employed was that used for classroom demonstrations at the Johns Hopkins Medical School. It was so arranged with pulleys and weights that a cord attached to the heel pulled a pointer by which the extent of the kick was recorded on a kymograph. The taps were administered by an electrical hammer. The technique, to be sure, was far from ideal but the conditions of the experiment were kept as constant as possible. For each individual the observation hour, the weight pulled and the arc through which the hammer fell were the same. From ten to fifteen contractions were recorded each day at regular intervals, the height measured in millimeters and the average taken. While the records were being made the subject closed her eyes and was asked to think of something definitely apart from the experiment. Although it became a matter of routine, no doubt such things as a desire to be through with the experiment or disquiet regarding a coming class or examination sometimes acted as a modifying influence.

Fourteen young women, college students and instructors, served as subjects. The ages ranged from eighteen to thirty-four years. They were in normal health and the menstrual periods caused no interruption in their regular college duties. Of the fourteen, five were finally discarded—one because of a systematic failure to give a response sufficiently strong to be accurately recorded, two others were so sensitive that the slightest tap caused a kick often too extensive to be registered with the apparatus used, while in the other two interruptions in the records at critical periods unfortunately decreased their value. On the other hand one subject (I) served as subject for three different periods, several months intervening between each; another (VI) at two periods with a four months interval; while in a third (IX) two years intervened between the observations. The records of these nine women included from one to four menstrual periods—thirty-three in all, their duration varying from four to seven days. The four days preceding and following the period have been uniformly considered as the pre- and postmenstrual intervals and the remaining days of the cycle as the intermenstrual phase.

Both morning and afternoon records were made from three subjects with the same general results but since those taken in the morning tended to be more regular, only a morning hour was adopted for the others of the group. The amplitude of the contractions for an observation period always varied—sometimes as much as from 60 to 70 mm.—though in general the tendency would be for all to be either high or low. Unaccountable changes naturally occur in the averages from day to day, for example taking a few successive days from the record of an intermenstrual period the averages in millimeters are 85, 93, 106, 53, 33, 35, 19. Curves were plotted from the daily records of each subject but the mathematical curves with fewer irregularities were more easily interpreted. Figure 1 gives such for subject II; above the daily record a summary of the days of each phase is given.

A consideration of the data for each individual has led to the preparation of the following summaries.

In subject I a rise occurs during four of the menstrual periods, depression precedes the onset and follows the cessation. In the other three the maximum increase does not appear until the postmenstrual phase.

In subject II two complete cycles were covered, a third cycle lacked the premenstrual and a fourth, the postmenstrual phase. In each instance a premenstrual rise is followed by a period of depression (fig. 1).

Two cycles in subject III show a premenstrual depression and a rise during the period but in a third the maximum is not attained until its cessation.

The record of IV shows nothing regular or significant except a slight depression during the two menstrual periods followed in one by a rise and in the other by continued depression.

In subject V a marked menstrual rise is preceded and followed by depression in the one cycle covered.

The record of VI, including almost five cycles, failed to give constant or significant results.

In VII the one outstanding point was the definite rise immediately following the cessation of the two periods.

A premenstrual rise is followed in VIII by a gradual decline which continues into the postmenstrual phase.

The first series of observations on IX, including four cycles, show nothing of significance but in a second series a premenstrual rise precedes three periods and in a fourth a slight increase accompanies it.

The general trend of the curves of these nine subjects may be more readily compared by examining figure 2, in which for each the averages of the observations made during all the days of the premenstrual, menstrual, postmenstrual and intermenstrual phases are shown. Summarizing the above results a significant change in the amplitude

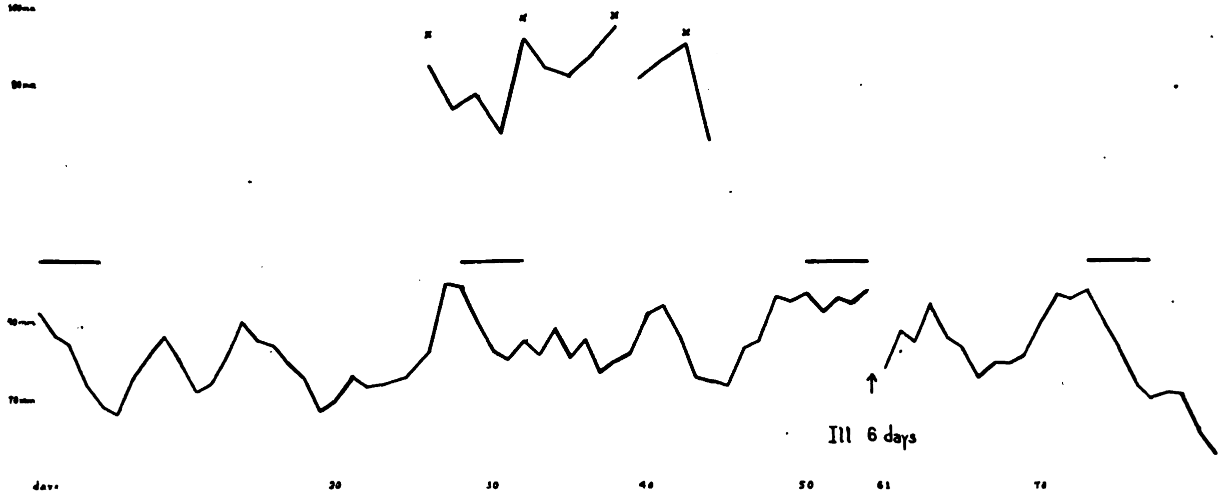


Fig. 1. Mathematical curve plotted from daily observations of the knee-jerk made on subject II. Each point is the average of from ten to fifteen readings. The menstrual phases are included under the horizontal lines. The shorter curve above represents the averages of the observations for all the days of each phase. The crosses indicate the menstrual averages.

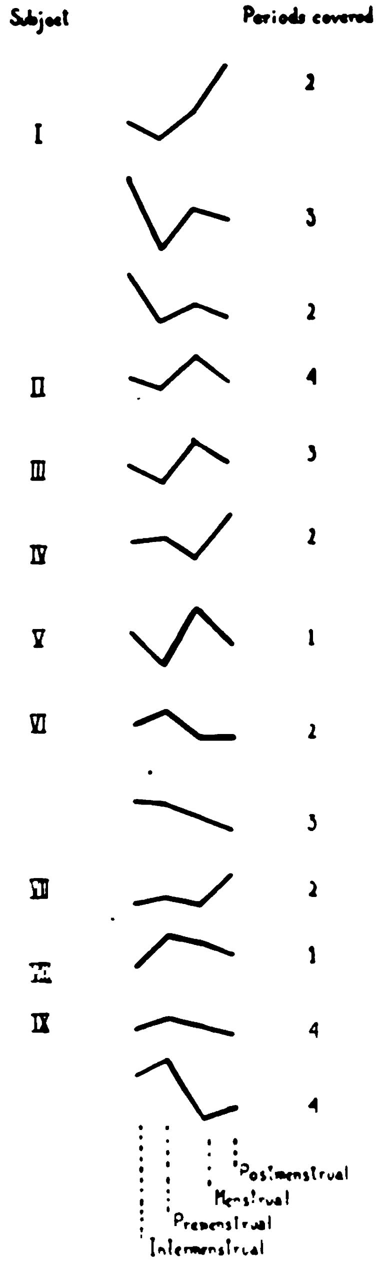


Fig. 2. Curves plotted from the averages of all the days included in the four phases for each of the nine subjects. (These curves are drawn on a smaller scale than those of figure 1.)

of the knee-jerk is shown in twenty-three instances, occurring in eight individuals. Eleven of these show a menstrual rise, six a rise immediately preceding the onset and six a rise at its close. Ten periods in four individuals show practically no change. In some instances a period of decided depression precedes the menstrual onset and in the majority of cases depression again occurs after the high point is reached, the tendency then being to approach an intermenstrual average which falls between the two extremes.

CONCLUSION

If the knee-jerk may be taken as a physiological index of the tone of the nerve centers, the evidence points in the direction of the following conclusions—that there is a period of hyperexcitability immediately preceding, accompanying or following menstruation and that there is then a tendency toward a fall and an approach to an intermenstrual average.

BIBLIOGRAPHY

(1) King: This Journal, 1914, xxxiv, 203.
(2) Sherman, Gillett and Pope: Journ. Biol. Chem., 1918, xxxiv, 373.
(3) Sherman, Wheeler and Yates: Journ. Biol. Chem., 1918, xxxiv, 383.
(4) Gillett, Wheeler and Yates: This Journal, 1918, xlvii, 25.
(5) Luciani: Human physiology, 1915, iii, 329.
(6) Dodge: Zeitschr. f. Allg. Physiol., 1911, xii, 1.
(7) Lombard: Amer. Journ. Psych., 1887, 1.
(8) Warren: Wood's Ref. Handb. of Med. Sciences, 1892, v, 363.
(9) Bowditch and Warren: Journ. Physiol., 1890, xi, 25.

ERRATA

[To be inserted between pp. 448 and 449, Vol. xliv.]

Page 449, line 20, for "$\frac{\theta_m \qquad 1}{0.825\ .\ \text{wt.}}$" read "$\theta_m \times$ sp. ht."
line 23, the equation should read, $1.3 \times 10^{-3} \times .83 = 11. \times 10^{-4}$ cal.
line 26, the equation should read, $9.2 \times 10^{-4} \times .83 = 7.6 \times 10^{-4}$ cal.
Page 450, the upper equation should read, $1.2 \times 10^{-3} \times .83 = 9.9 \times 10^{-4}$ cal.
In the second equation for "4.8" read "11.2," and for "1.3" read "3.0."
In the second line above the italicized paragraph for "1.55" read "3.6," and for "4.2" read "9.8;" in the paragraph itself for "0.155" read "0.36," for "0.042" read ".098."
In the seventh line from the bottom for "a gratifying agreement with" read, "a number nearly one-half of".
Page 451, line 1, for "ten", read "four".
line 9, delete entire paragraph.
Page 452, line 8, for "0.00048" read "00.10."
line 10, for "0.155" read ".36."
line 12, for "4.2" read "9.8."

ERRATA

[To be inserted between pp. 158 and 159, Vol. xlvii.]

Page 159, line 15, for "0.0007" read ".0018,"
Page 160, line 4, for the sentence beginning here read, "The heat produced was .83 × .00156, or .0013 calorie per gram ventricle."
Page 163, line 19, for "0.00048, 0.00052 and 0.00073", read ".0011, .0013 and .0018."
line 21, for "0.00062" read, ".00155"
line 30, for "1.3" and "1.67" read "3.2" and "4.5."
Page 164, last line, for "well within" read "nearer the greater of."

---

electrical deflections.

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 47 JANUARY 1, 1919 No. 4

STUDIES ON THE SUBMAXILLARY GLAND

I. ELECTRICAL DEFLECTIONS IN GENERAL[1]

ROBERT GESELL

From the Department of Physiology of the Washington University School of Medicine, St. Louis

Received for publication October 26, 1918

GENERAL AND INTRODUCTORY

The object of this research is to gain more data on the significance of electrical deflections[2] of living tissue.

The work here reported represents data obtained from experiments on the submaxillary gland of the dog. The choice of the submaxillary gland for an object of study is obvious. In the first place, it is a small compact mass of tissue lying near the surface and easily dissected. It has two sets of nerves differing considerably in function, likewise easily accessible. The volume-flow of blood may be controlled mechanically and by nerve stimulation and this flow measured. Electrodes can be easily and more or less uniformly applied. The work of the gland may be quantitatively controlled and measured. These advantages have appealed to many for study of phenomena in living tissue and consequently there is a very considerable literature on the physiology of the salivary glands at our disposal. This is of no small importance as regards the interpretation of such a variable factor as electrical deflections.

---

[1] Reported before the American Physiological Society 1916 and 1917. Proceedings in this Journal, 1917, xlii, 591 and 1918, xlv, 559.

[2] The term electrical deflection is preferred to that of electrical variation. The term electrical variation will be employed only to describe variations in electrical deflections.

In reading the literature[3] on electrical physiology of glandular structures one is impressed with the variability of deflections obtained even when the tissues presumably are activated and treated in the same manner. The difficulty of explaining these variations, particularly the complete reversal of deflections, has been encountered by many. Unfortunately in the study of electrical physiology of glandular structures the graphic method has been little employed. Visual observations of the deflections of the galvanometer sufficed to supply the data. A trial of this method demonstrated the difficulty of gaining a clear picture of the electrical deflections, let alone the time relation of synchronous phenomena in the gland. Therefore it seemed important to use a graphic and continuous method with which every deflection in the course of an experiment can be recorded. The use of the photographic method with the string galvanometer as employed by Schaeffer, and with the d'Arsonval galvanometer as developed by Cannon, were considered but the synchronous photographing of chorda stimulation, of sympathetic stimulation, of secretion, volume-flow of blood and electrical deflections seemed too cumbersome and difficult. In addition, if a large number of experiments are performed the method becomes expensive, but most important is the inability to follow the progress of the experiment. A picture of the results is available only after the experiment is completed. The importance of knowing at the time when constant deflections are obtained so that a new variable might be introduced became evident in the course of the research and proved to be a most important factor.

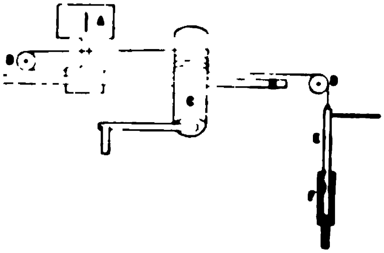


Fig. 1. Device for recording deflections of a d'Arsonval galvanometer on smoked paper. *A*, sliding target for following the reflected filament; *B*, guide for sliding target; *C*, windlass operating the target; *D*, pulleys; *E*, weighted shaft carrying writing point; *F*, guide for weighted shaft.

A graphic method of recording deflections of a d'Arsonval galvanometer on smoked paper was developed. With this method the employment of controls was an easy matter. While using the method I learned that Spaeth employed the same principle for recording the movements of melanophores, and more recently, that the method has been in use in psychological laboratories for recording electrical deflections. But the device developed proved of such value in this research that it is shown in figure 1. The deflections are followed by an assistant. A horizontally sliding target, operated by a windlass, is kept in line with the horizontally reflected filament of the galvanometer. Attached to the target by thread is a vertically moving writing point which records the deflections. With the type of galvanometer[4] used the deflections were easily followed. Though at times these deflections are rapid and require some skill to follow promptly, the lag is only momentary and imperceptible with the slowly revolving drum usually employed.

---

[3] The review of literature is reserved for later papers.

By the use of this method, employed continuously throughout an experiment, a partial explanation of the variability of deflections was made possible. That some of these variations are more apparent than real is shown.

The Williams box proved most convenient for controlling the compensating current. The non-polarizable electrodes were the usual cotton, filter paper variety.

The electrical deflections accompanying glandular activation and recovery were continuously recorded. The gland was most commonly activated by stimulation of the chorda tympani, by stimulation of the sympathetic fibers alone or with the vagus fibers, and by injection of pilocarpin. The effects of adrenin, pituitrin, atropin, saline solution, etc., and of mechanical interference of the blood supply to the gland or interference to the flow of saliva through the duct were also determined.

To assist in the interpretation of the accompanying deflections, various synchronous records were made: blood pressure, volume-flow of blood by an automatic and bloodless method, salivary secretion, time in seconds, time and duration of stimulation, of injections, of change in blood supply and of obstruction of the salivary duct.

Two types of leads were employed the "two gland lead" and the "one gland lead." With the two gland lead both glands are exposed and an electrode placed on the outer surface of each. This arrangement suffices for studying effects of procedures limited to one gland only, such as stimulation of the glandular nerves on one side or obstruction of the salivary duct. If the effects of activation are bilateral, as with injections, isolation of one gland by a previous mass ligature is

---

[4] The galvanometer used in this research is made by the Leeds & Northrup Company. They give the following description in bulletin 228.—Galvanometer Resistance Ohms 550, Sensitivity 10,000 megohms = 10^{-10} amperes, Period Seconds 17.5, Critical damping resistance, external, ohms, 20,000.

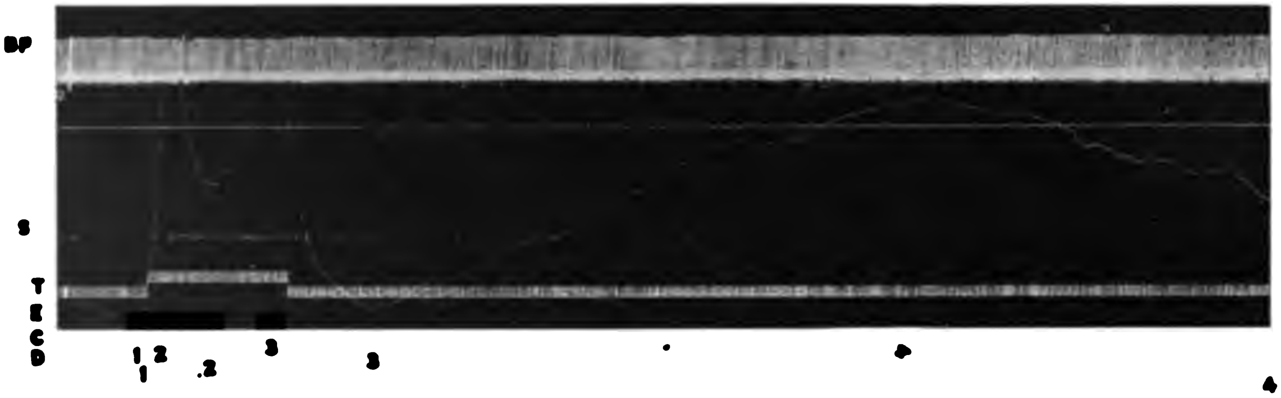


Fig. 2. An electrical deflection obtained with prolonged chorda stimulation, showing the four crests and four depressions, the four ascending limbs or negative components and the four descending limbs or positive components. *B.P.*, blood pressure; *S*, salivary secretion; *T*, time in seconds; *E*, electrical deflection. The entire deflection required about eighteen minutes for its completion.

necessary. Such a ligature initiates changes in the gland, disturbing the electrical equilibrium for some time. But if this ligature is made at the beginning of the operation equilibrium is usually established by the time observations of electrical deflections begin.

When the "one gland lead" is employed both electrodes are placed on the activated gland, one usually on the hilus and the other on the outer surface opposite the hilus or a point slightly posterior to it.

With the "two gland lead" the electrodes are always placed so that an upward deflection indicates an increased negativity of the lead on the activated gland (the active lead) with respect to the lead on the resting or dead gland (the indifferent lead). With the one gland lead the hilus is usually the constant lead (exceptions to this will be noted). Upward deflection indicates the outer surface of the gland to be electrically negative to the hilus surface.

Each type of lead gives a more or less characteristic deflection—sometimes very similar, depending upon the points of the gland led from. But even though the general appearance of the deflections be dissimilar, careful study of the crests and depressions will show them to be comparable.

A typical deflection, with the fundamental crests and depressions, obtained by chorda stimulation of about sixty seconds duration with a "two gland lead," is shown in figure 2. It is composed of four negative waves or crests, 1, 2, 3 and 4, and four depressions, 1, 2, 3 and 4. The upstrokes are designated as negative components 1, 2, 3 and 4, and the down strokes as positive components 1, 2, 3 and 4.

At the beginning of this research the "two gland lead" alone was employed and with it a degree of similarity of deflections was obtained which gave promise of approaching a uniformity of results necessary for interpretation of the deflections, yet not infrequently marked dissimilarities occurred. Therefore the problem was to determine the nature of these unknown variables in order that only one new and known variable at a time might be introduced for study.

The maintenance of the uniform condition of the animal, a uniform application of the leading off electrodes and the uniform application of the stimulating electrodes proved very important in reducing the variable factors. This is evident, for a single deflection may last as long as twenty minutes and such deflections separated by an hour or more are frequently compared.

In addition to the variability of the individual deflections we often have a continual shifting of the electrical base line, the electrical condition of rest. This shifting of the base line has a double source. As in the case of the variability of deflections, it also is dependent upon uniformity in the condition of the tissues and in the application of the leading off electrodes.

The most important factor in keeping the condition of the animal constant is uniform anaesthesia which was accomplished by the administration of a relatively large injection of morphine sulphate. The amount of ether given was usually adjusted at the beginning of the experiment and kept at that adjustment to the end for it was noted that a sudden change in the etherization often produced an electrical deflection.

The other source of change in the base line is at the leading-off electrodes—attributable to either shifting of position or to drying. A considerable shift in the position of the gland is without effect on the base line provided the wisp of cotton of the electrode moves with the gland. Electrodes mounted on rods of block tin and properly applied, together with sufficient anaesthetization to prevent the larger movements overcome this difficulty. A common source of shifting of the base line is the drying of the electrodes but contacts consisting of heavy wisps of cotton, frequently moistened with saline prevent such shifting.

Uniformity of application of the stimulating electrodes is absolutely necessary in quantitative work and for comparison of a series of deflections with long intervening periods. By employing certain precautions uniformity of stimulation is obtainable. The chorda lingual nerve, dissected to the maximum length, with all the branches cut excepting the chorda tympani, was placed in shielded electrodes mounted on rods of block tin and protected by moist cotton. Injury to the chorda tympani fibers was prevented and diffuse stimulation secured by placing the thickest part of the nerve in the electrodes and by using large animals. The cut end of the nerve was weighted with thread and lead weight so as to maintain constant pressure against the electrodes.

Presumably any change in the metabolic processes of a tissue is accompanied by some electrical response. The factors which may influence the processes of the submaxillary gland are therefore very numerous. Strength of stimulation of the nerves leading to the gland, duration of stimulation, period of rest between periods of stimulation, variations in the volume-flow of blood, resistance to the flow of saliva, injection of pilocarpin, adrenin, atropin, etc., are among those studied.

In this paper only data of general interest are considered. A more detailed study forms the basis of another paper.

Activation of the gland by stimulation of the chorda tympani is the most perfectly controllable means of altering the processes in the gland. It is the only method used in the expreriments described in this paper which deals with the effects of, *a*, duration of stimulation; *b*, strength of stimulation; *c*, period of rest between periods of stimulation; *d*, exercise, and *e*, leads.

In studying the effect of varying any one of these factors it is necessary to know that the particular change introduced is the only, or at least the main, factor varied. This can be the case only if there are no slowly progressing changes in the animal as a whole or in the gland itself as a result of activation. It is therefore necessary to make control observations to determine whether any unknown variable is operating. In making these controls conditions were kept as constant as the methods permitted, i.e., a constant lead was employed, the chorda lingual nerve stimulated the same length of time, with the same strength of stimulus and with equal periods of rest intervening. If similar deflections were obtained with similar activation as indicated by the number of drops of saliva and the electrical deflection, it was assumed that conditions were constant enough for introducing a variable. Very frequently at the beginning of an experiment the gland reacted differently for a period of an hour or more, but occasionally equilibrium

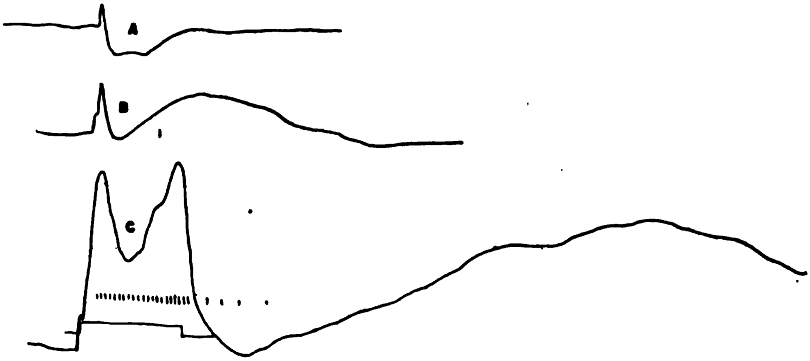


Fig. 3. Deflection showing the effect of varying the duration of stimulation. Secretion in drops is shown in curves *B* and *C*. In curve *A*, the stimulus was too short to elicit secretion. Crest number four is only partially shown in figure 3, *C*.

of the base line and uniformity of deflection were established in a short time.

a. Duration of stimulation. By keeping constant the lead, the strength of stimulation and the period of rest between periods of stimulation, the effect of varying the duration of stimulation was studied (see fig. 3). The lead in this case was a "one gland lead," one electrode resting on the hilus of the activated gland and the other approximately opposite this point on the outer side of the gland.

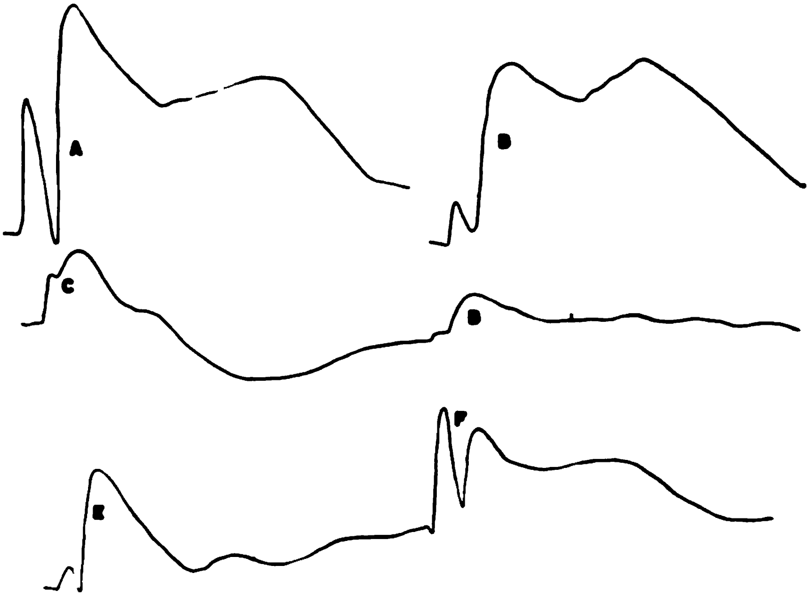


Fig. 4. Deflections showing the effect of changing strength of stimulus. The lead is constant, the period of rest five minutes, the period of stimulation twenty seconds. The strength of stimulation is progressively decreased from *A* to *D* and again increased from *D* to *F*.

With a very short stimulus of about a second's duration, crest number 1 alone may appear as a low sharp wave. Frequently this is followed by a low slowly developing wave, which may be crest number 4 (see fig. 3, *A*). By prolonging the stimulation, crest number two is added and the appearance of crest number 4 made more certain (fig. 3, *B*). With the addition of crest number 2, crest number 1 may not always be as distinctly seen. This depends on the rapidity of the development of the second negative component. If this component develops strongly before the first negative component has completed its effect upon the galvanometer and before the first positive component comes into play, the first and second negative components may be more or less completely fused. In certain experiments the second negative component may develop so slowly that the first positive component reaches the base line and separates crests 1 and 2 completely. In figure 3 fusion is only partial. Though many curves show complete fusion component number 1 can be elicited by special methods in every animal. If the stimulus is prolonged still more than in 3 *A* and *B*, crest number 3 is added and crest number 4 is usually enlarged (fig. 3, *C*). This is the common picture produced by prolonged chorda stimulation; the original is shown in figure 2. There are, however,

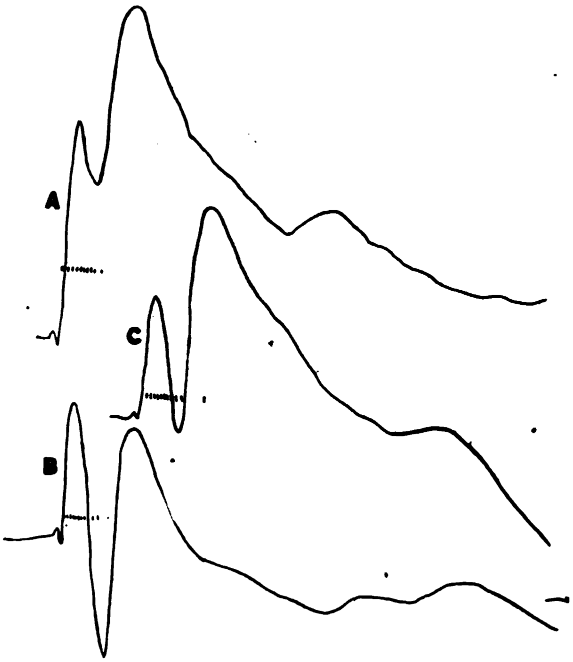


Fig. 5. Deflections showing the effects of rest. Strength of stimulation was constant, the duration of stimulation was twenty seconds in each observation. The period of rest preceding deflection *A* was 30 minutes; *B*, 5 minutes and *C*, 15 minutes. Though the same amount of secretion is elicited in each case the deflections vary but approximate each other with the approximation of the period of rest.

certain exceptions—additional waves may appear and crest number 4 may be absent. These will be considered in a later paper.

b. Strength of stimulus. By keeping constant the duration of stimulation, the duration of rest and the lead, the effect of varying the strength of stimulation may be studied, as shown in figure 4. In these observations the strength of stimulation is progressively diminished from *A* to *D* and increased again to *F*. A changing contour with diminishing strength of stimulation and the approximate duplication of curves on increasing the strength of stimulation, such as is seen here, occurs even though two instead of one variable may be introduced in this experiment. The difficulty of confining the variables to strength

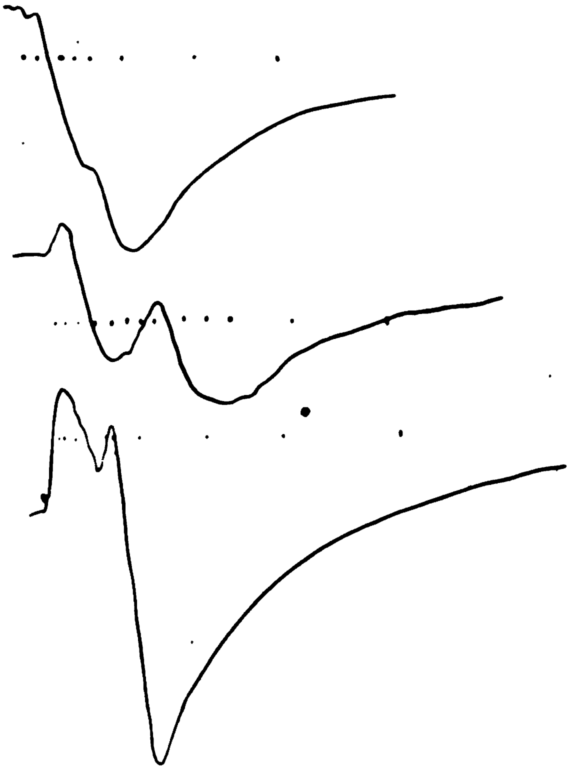


Fig. 6. Observations showing a changing secretory response to stimuli of equal strength and duration applied at equal intervals, with the development of reversal of the electrical deflection.

of stimulation alone is apparent, for a period of rest following a period of strong stimulation is relatively shorter than a period of rest following a weaker stimulation. In these observations, however, strength of stimulation appears to be the important variable.

c. Duration of rest. The effect of rest is shown in figure 5, three observations in which the lead, the duration and strength of stimulation were constant but the rest preceding such stimulations variable. The period of rest preceding observation *A* was thirty minutes; observation *B* five minutes; observation *C* fifteen minutes. The change in contour with changing rest and the approximation of contour with approximation of the period of rest become obvious upon comparing deflections *A*, *B* and *C*. This change in contour is particularly interesting in view of the fact that the amount and rate of secretion remain the same. In the two preceding experiments on the effect of duration and of strength of stimulation, the contour of the deflections may be largely accounted for by the changing rate and amount of secretion. These factors are however missing in the present instance. The difference in the deflections accompanying equal secretions may possibly be looked upon as an effect of altered time relation between such processes as liberation and elaboration of secretion resulting from altered periods of rest.

d. Exercise. Occasionally, as in the preceding experiment, a previous stimulation of the chorda tympani has little or no noticeable effect upon the amount of secretion obtained by subsequent stimulation. Not infrequently, however, when a series of stimuli, of equal strength and duration with equal periods of rest, is applied to the chorda lingual nerve the preceding stimulus has its effect on the subsequent stimulus. This has been looked upon as an effect of exercise. When the electrical deflections have been recorded in such observations changes in contour occurred. (See fig. 6.) The general contour of the curves varies considerably, producing what is known as a reversal of the deflection, but a more careful study shows that these variations are more apparent than real—that the fundamental crests appear in each deflection and that it is only their amplitude and positions which are altered by a change in the balance of the adjacent negative and positive components. To what extent these changes in balance are due to the effect of exercise and how much to the increased response of the gland to stimulation, it is difficult to say. But the observation is of interest in that it shows a difference in response to stimulation and also that it shows the gradual development of a so-called reversal.

The observation illustrates the difficulty occasionally met with in interpreting deflections and in securing proper controls.

e. Lead. The two general types of leads—"one and two gland leads"—have already been described, and mention has been made of the fact that the deflections obtained with these two leads may resemble each other closely. To facilitate the description of various leads, certain points on the gland are numbered from 1 to 8. (See fig. 7). Numbers 1, 2 and 3, are on the outer side of the gland, 4, 5, 6, 7 and 8 on the edge of the gland between the outer and inner surfaces. The points corresponding to 1, 2 and 3 on the inner surface of the gland are numbered 1′, 2′ and 3′; 2′ corresponds to the hilus of the gland. If the lead is a "one gland lead," the leads coming from the hilus and a point directly opposite on the outer surface, it is designated lead 2′ - 2. Occasionally the lead may not be over a numbered point; e.g., it may be between 2 and 3. In that case the lead is designated as lead 2′-(2-3).

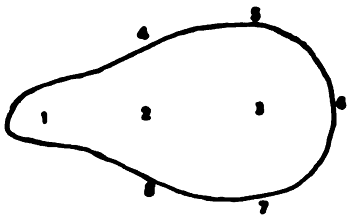


Fig. 7. Schema of the submaxillary gland showing leads.

If the lead is a "two gland" or a symmetrical lead, one electrode (the indifferent electrode) on the dead or resting gland and the other, e.g., on point 2 of the activated gland, the lead is designated as lead S - 2.

In figure 8 is shown a series of deflections obtained with a two gland lead resulting from chorda stimulations of equal duration (10 seconds) and equal intensity, the points of application of the electrodes being designated for each deflection. The deflection changes distinctly with each lead, more with some than with others, e.g., in observations *A*, *B* and *C* (leads S - 2, S - 1, and S - 2′), the electrical variation is greater than in observations *C*, *D* and G (leads S - 4, S - 5 and S - (5 - 6)). Each deflection is typical for the lead for when pairs of observations are taken for each lead the deflections in each pair show a close resemblance to each other, which is absent in deflections from different leads. Or if one is fortunate enough to keep conditions very constant, with careful application of the electrodes, duplicate series may be produced. In figure 8, deflections *A* and *F*, and *C* and *G* (each pair having approximately the same lead) resemble each other most closely even though separated by a considerable interval of time. (With observations *A* and *F* the leads presumably are the same; with observations *C* and *G*, the leads are only approximately the same).

Figure 9 shows a series of deflections obtained with a "one gland lead." The typical difference between a one and two gland lead is brought out fairly well by comparison of figures 8 and 9. In the series of figure 9, even though the lead was changed for each subsequent observation, there is an approximate duplication of the deflections when the leads are repeated as in observations *B* and *H*. (Leads 2′ – 1), *E* and *F* (leads 2′ – 3), and *D* and *G* (leads 2′ – (3′ – 6)).

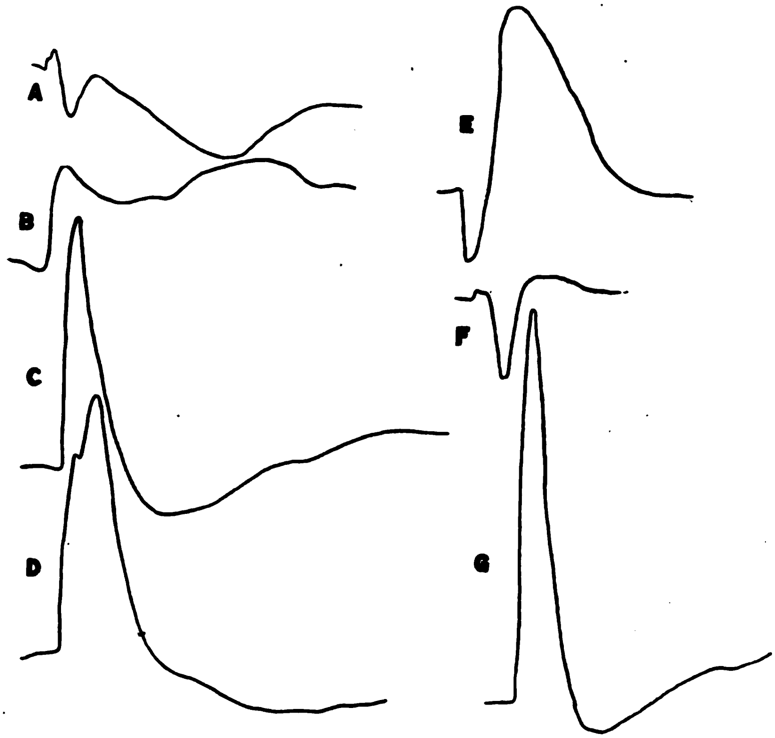


Fig. 8. Deflections showing the effect of changing leads. The curves are obtained from "two gland leads, "the lead on the dead gland remains constant, the lead on the activated gland is shifted as designated in the figure. *A*, S – 2, *B*, S – 1; *C*, S – 5.5; *D*, S – 4; *E*, S – 2′; *F*, S – 2; *G*, S – 5.

The observations represented in figures 8 and 9 illustrate the importance of knowing definitely the lead employed if an interpretation of the deflection is to be made or the observations of different workers are to be compared. Though in the "two gland leads" the deflections are mainly above the electrical base line of rest, in the "one gland leads," large deflections below the base line occur. The extent to which this occurs in the "one gland" lead differs with the points led off. For example, deflection *B*, when compared with deflection *A* (fig. 9), might be considered a complete reversal yet the processes represented by the deflections are the same. Therefore a description of a reversal of the usual deflection without mention of the lead means little. The reversal with a constant lead as seen in figure 6 however, is of interest.

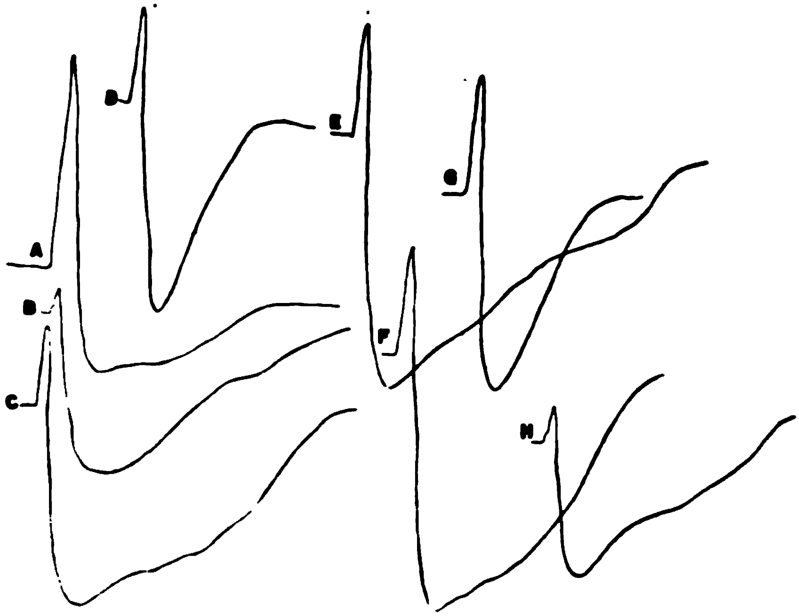


Fig. 9. Deflections showing the effect of changing leads. The curves are all obtained from "one gland leads," the 2′ or hilus lead remaining constant. *A*; 2′– 6; *B*, 2′ – 1; *C*, 2′ – 2; *D*, 2′ – (3′ – 6); *E*, 2′ – 3; *F*, 2′ – 3; *G*, 2′ – (3′ – 6). *H*, 2′ – 1.

f. Duration of stimulation, strength of stimulation, period of rest and leads constant. From the observations so far cited it is apparent that the electrical deflections are attributable to the nature and the sequence of processes going on within the gland and to the type of lead employed to register the electrical changes accompanying these processes. That comparable control deflections can be obtained was pointed out in the beginning of the paper and now that the effect of varying a number of factors has been shown it might be of interest to know how constant the conditions in a gland may be kept and how accurately these conditions are reflected in the electrical deflections.

If we have an animal whose condition remains constant and if we employ a series of stimuli of equal strength and duration separated by equal periods of rest, the nature and sequence of the secretory and recovery processes should be the same provided the period of rest is long enough for recovery and the effects of exercise do not enter. If these conditions are met one might expect the electrical deflections to be constant with a constant lead. It is only if this is true that the electrical deflections may be considered a reliable index to glandular processes and that interpretations of the deflection become possible.

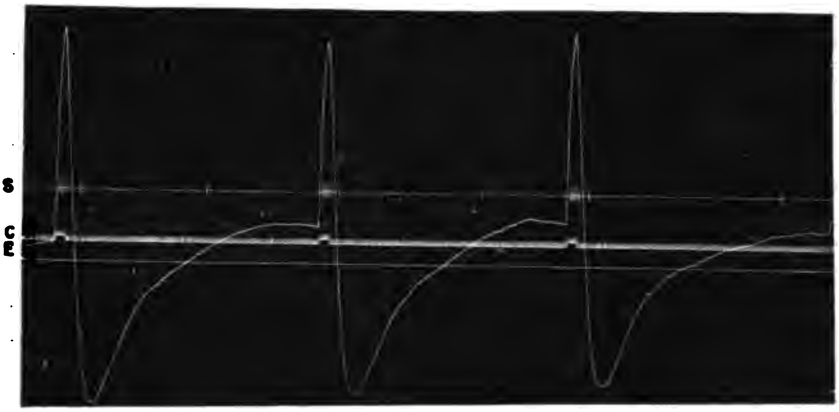


Fig. 10. Deflections obtained under constant conditions with strength and duration of stimulation and the intervening periods of rest equal.

In the series of observations shown in figure 10 the chorda tympani nerve was stimulated in each case for 8 seconds with stimuli of equal strength, a constant period of rest of 5 minutes intervening. In each case exactly eight drops of saliva were secreted. The deflections are practically superimposable and reflect the constancy of intensity and sequence of glandular processes.

Knowing in a general way the significance of some of the more important variables we are better prepared to study in detail certain factors influencing glandular function and, therefore, the electrical responses.

It will be noted in figure 2 that secretory fluctuations run parallel with crests 2 and 3. To study the relation of these and other phenomena to blood-flow an automatic and bloodless method of recording the volume-flow of blood was devised and is described in paper II. In figure 3 of that paper a record of volume-flow of blood with prolonged chorda stimulation is shown. Paper III deals with the nature of the fluctuation in flow there noted, with the effect of volume-flow of blood on metabolism, the effect of metabolism on volume-flow of blood.

SUMMARY

The object of this research was to study the significance of electrical deflections in living tissue.

The submaxillary gland was chosen as a type of tissue easily accessible for study and with functions well controllable.

The gland was activated by stimulation of the chorda tympani fibers, under varying circumstances, with stimuli of varying intensity and duration. The associated deflections were recorded.

On account of the great variability of electrical deflections common to glandular structures, a continuous and graphic method of recording these deflections on smoked paper was employed.

The advantages of this method over the photographic method are discussed. It permits the recording of every deflection, the establishment of control observations and shows the results during the progress of the experiment.

To aid in the study of these deflections, blood pressure, salivary secretion, volume-flow of blood and time in seconds were synchronously recorded.

A prolonged chorda stimulation of about sixty seconds duration usually produces a deflection of four crests, the result of a balance of four negative and four positive components.

Though most deflections are comparable in their essential points, employing a single type of lead and activating the gland presumably under constant conditions elicits deflections varying in general contour.

To determine the unknown variables producing these electrical variations special precautions were taken.

By keeping the general condition of the animal constant, using a constant lead and activating the gland with stimuli of equal duration, equal intensity and separated by equal periods of rest, it was possible to obtain superimposable deflections.

With the aid of such controls it was shown, by varying single factors, that the contour of the electrical deflections may be decidedly altered by change of strength of stimulation, of intensity of stimulation, of leads, of the period of rest and by the factor of exercise.

Where the leads are constant the electrical variations may be attributable to changes in intensity and sequence of such processes as liberation of secretion, elaboration of secretion, recovery, etc.

The cardinal points of a typical deflection are usually present, though the balance of components may change markedly the general contour of the deflection.

This contour may change to what is known as a reversal. If the change in deflection occurs with a constant lead it is of significance. If it occurs as result of an altered lead it is of no value in indicating altered glandular processes.

This research is considered only as a necessary preliminary to further detailed study.

STUDIES ON THE SUBMAXILLARY GLAND

II. An Automatic and Bloodless Method of Recording the Volume-flow of Blood[1]

ROBERT GESELL

From the Physiological Laboratory of Washington University School of Medicine, St. Louis

Received for publication October 26, 1918

In the study of electrical variations of the submaxillary gland, I found that an interpretation of these variations required a continuous record of the volume-flow of blood through the gland.

Though the electrical variations are purely local reactions, they nevertheless are influenced by fluctuations of the general condition of the animal and for that reason it is essential to keep this condition as uniform as possible. Since at times it is necessary to compare deflections separated by intervals of an hour or more, the usual methods of recording the volume-flow of blood would introduce serious difficulties. The rigid requirements of the experiment suggested the importance of developing a new method for recording the flow of blood.

In the method which was developed the blood, without coming in contact with any foreign substance, is automatically measured as it flows, at approximately zero pressure, through the vein toward the heart. (See figs. 1 and 2.)

In measuring the volume-flow of blood of the submaxillary gland, *1*, the jugular vein, *2*, is cleanly dissected. Every branch with the exception of the branches coming from the gland is ligated. The vein is then placed in the trough, *3*, of the instrument under the emptying-plate, *4*, and under the cut-off, *5*, which rests upon an extension of the floor of the trough. The emptying-plate and cut-off are pivoted at *6* and *7* and are operated respectively by the emptying solenoid, *8*, and the cut-off solenoid *9*. The cut-off is held down on the vein by the constant pull of the spring, *10,* attached to the frame of the cut-off solenoid. The emptying-plate is perfectly balanced and free to swing in a vertical direction.

---

[1] Reported before the American Physiological Society. Proceedings, this Journal, 1918, xlv, 545.

This arrangement permits the automatic filling and emptying of the vein lying in the trough. With the vein empty and the cut-off closed, the vein is filled by the blood coming from the gland and raises the emptying-plate until the cut-off contact, *11,* is made. This contact closes the circuit of the cut-off solenoid, the iron core, *12,* attached by a piston, *13* (to be described) to the cut-off, is drawn down against the pull of the spring, *10,* and the cut-off opens, permitting the blood collected under the emptying-plate to pass to the heart. When the vein collapses, the emptying-plate follows and the cut-off circuit is broken. The cut-off closes the vein until the cut-off contact is again made by the refilling of the vein. In this way the vein automatically fills and empties at a rate dependent on the volume-flow of blood.

This describes the operation of the instrument in its simplest form in which the cut-off solenoid alone is used. Though records of volume-flow of blood are obtainable with an instrument of this kind, a few modifications were necessary to perfect the method and to make it yield quantitative data.

In the instrument without an emptying solenoid, the vein is filled against a positive pressure (weighted emptying-plate) which assists in emptying the vein during the period in which the cut-off is open. The blood however has considerable viscosity and inertia, and therefore a constant weight which can be employed on this emptying-plate is insufficient to empty the vein completely during the short period that the cut-off is open. In addition respiratory variations of venous pressure continually obtaining on the cardiac side of the instrument vary the degree of emptying of the vein, resulting in a very irregular record. This irregularity may, however, be overcome by applying to the emptying-plate a force of sufficient strength to make the venous pressure oscillations negligible. But even so the resistance of the blood is sufficient to permit different degrees of emptying of the vein if the short period during which the cut-off is open varies only a fraction of a second. It was therefore necessary to prolong the open period of the cut-off as well as to apply a strong emptying force to the vein during this period. By prolonging the open period of the cut-off beyond that necessary to obtain complete emptying of the vein, it is unnecessary to have these periods of constant duration.

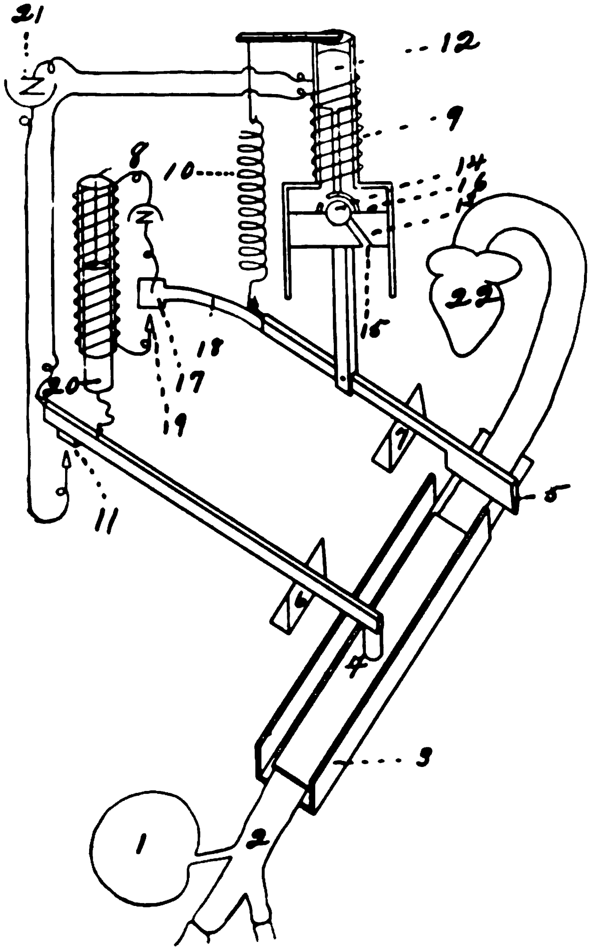


Fig. 1

Figs. 1 and 2. Schema and photograph of the automatic and bloodless volume-flow recorder. *1*, submaxillary gland; *2*, external jugular vein; *3*, trough; *4*, emptying-plate; *5*, cut-off; *6*, pivoting point of the arm of the emptying-plate; *7*, pivoting point of the cut-off arm; *8*, emptying solenoid; *9*, cut-off solenoid; *10*, closing spring of cut-off; *11*, cut-off contact; *12*, core of cut-off solenoid; *13*, cut-off piston; *14*, ball-bearing valve; *15*, opening communicating between exterior and piston; *16*, air space of cylinder; *17*, platinum plate; *18*, spring-brass strip; *19*, emptying-solenoid contact; *20*, core of emptying solenoid; *21*, batteries; *22*, heart; *23*, relay.

This period is prolonged by a piston with a ball valve, *14*, attached to the cut-off core and to the cut-off arm. The opening, *15*, connects the exterior and the air space, *16*, of the cylinder and casing of the core. The ball is freely movable and rests over the communicating opening. This arrangement permits the free entrance of air into the cylinder and a quick downward movement of the piston is possible, but at the end of the stroke the ball again closes the opening and the piston can now rise only if the air above escapes. The escape takes place through an escape valve not shown in the diagram. By the regulation of this valve together with the regulation of the closing spring the rate of closure of the cut-off is controlled. During the entire open period of the cut-off, a force is applied to the emptying plate by means of solenoid *8*, which brings about complete emptying.

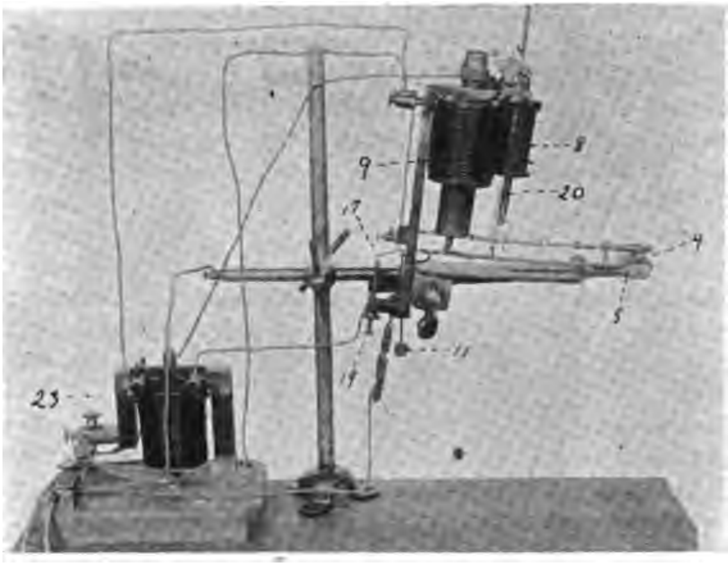


Fig. 2

From the previous description it is apparent that the prolongation of the open period is dependent on the length of the down stroke of the cut-off core. Though a solenoid may exert a powerful pull when a continuous current flows, this pull may be very small when only a temporary closure occurs. The duration of the closure of the cut-off contact is so short that it is necessary to prolong in a mechanical way the period of flow of current through the cut-off solenoid. This is accomplished by placing a specially constructed relay in the cut-off circuit. The solenoid of this relay is powerful, the core is heavy, and by mounting one of the platinum contacts on a flexible strip of spring-brass attached to the core, the bending of the strip against the second contact on each closure secures the necessary prolongation.

Aside from these modifications the instrument works on the same general principles as the simpler instrument. With the vein empty and the cut-off closed, the emptying plate rises until the cut-off contact is made. But now, when the cut-off opens, a platinum plate, *17*, mounted on a flexible strip of spring brass, *18*, is bent down on the emptying solenoid contact, *19*, closing the circuit of the emptying solenoid. The iron core, *20*, suspended in the solenoid and attached to the arm of the emptying plate by a thread or a brass spring, is drawn up and exerts its force on the emptying plate so long as the contact of the emptying solenoid is made. This contact is prolonged by the flexibility of the spring-brass strip and by the retardation of the closing of the cut-off. The height of the contact is so adjusted that the contact is broken only when the cut-off is completely closed. The breaking of the contact releases the force on the emptying plate and the vein is free to fill again against zero pressure, for the emptying plate is balanced by the long arm connected with the emptying solenoid.

When the emptying-plate compresses the vein, there is a tendency for the blood to be expressed in both directions. This is overcome by giving the plate a very slight tilt which sends the bulk of the blood in the direction of the heart.

The volume-flow is graphically recorded by a signal magnet in circuit with one of the solenoid circuits. Figure 3 is a record of the volume-flow of blood of the submaxillary gland showing the effect of stimulation of the chorda tympani. The number of strokes per unit of time may be regulated to meet the demands of the experiment and the volume-flow of blood obtaining With a rapid flow of blood a coarse adjustment is usually best while with a very slow flow a fine adjustment of the cut-off contact shows the changes in volume-flow more quickly. These adjustments are easily made by the screw showing in the photograph (fig. 2). Raising this contact diminishes the degree to which the vein fills in each interval. For qualitative work this adjustment may be freely used but for quantitative work it is necessary to calibrate the vein for every adjustment; and in doing

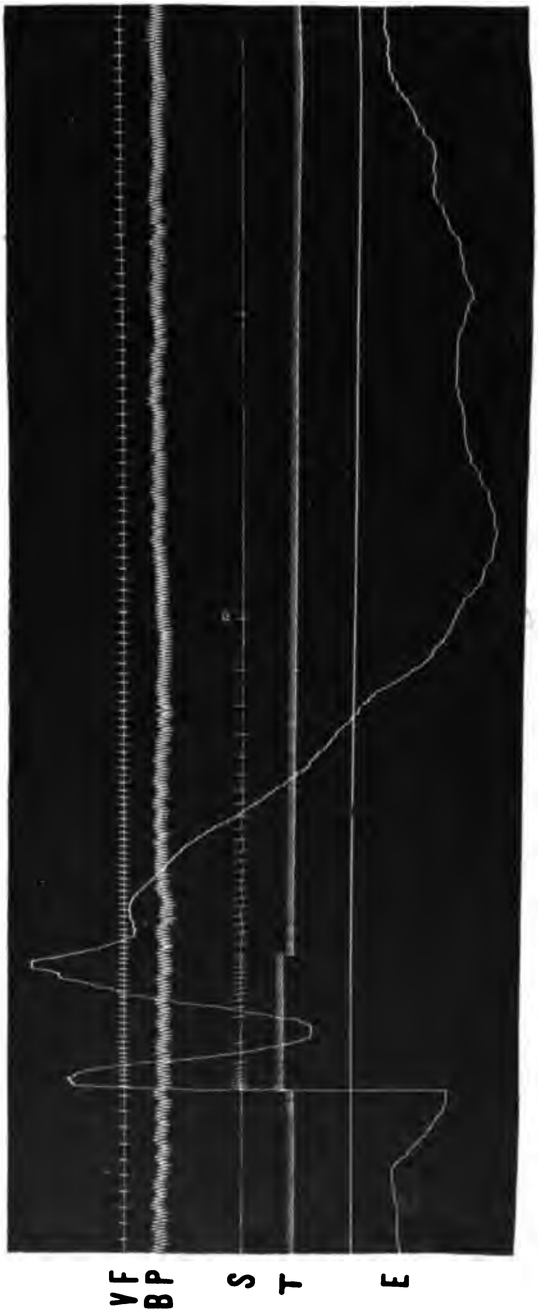


Fig. 3. Record showing volume-flow of blood from the submaxillary gland. *V.F.*, volume-flow of blood; *B.P.*, blood pressure; *S.*, salivary secretion in drops; *T.*, time in seconds, *E.*, electrical deflection.

quantitative work it is therefore desirable to set the adjustment at some mean position suitable for all conditions of the experiment.

The principle on which the instrument is constructed should permit quantitative measurements if the emptying solenoid contact is properly adjusted, and this is an easy matter. In this case all the blood passing through the vein is measured, for the vein is never completely open. It is always closed either at the lower end of the trough by the cut-off or at the upper end by the upper edge of the emptying-plate.

That the number of strokes in the record of volume-flow varies in direct proportion to the volume-flow of blood was demonstrated in two ways: (1) The external jugular vein was isolated and connected with a burette containing saline solution. Records were then made of volume-flows of known quantities of saline solution. The number of strokes of the instrument was directly proportional, within an error of about 2 per cent, whether the flow was rapid or slow. (2) The other method consisted in measuring the volume-flow of blood through the vein in situ by the bloodless method, and in measuring it again by collecting the blood after passing the instrument. For this purpose the blood is permitted to flow from the vein through a syringe needle and rubber tube, and the drops are recorded (see fig. 4). By varying the volume-flow of blood in various ways, it was found that the results obtained by the two methods agreed perfectly.

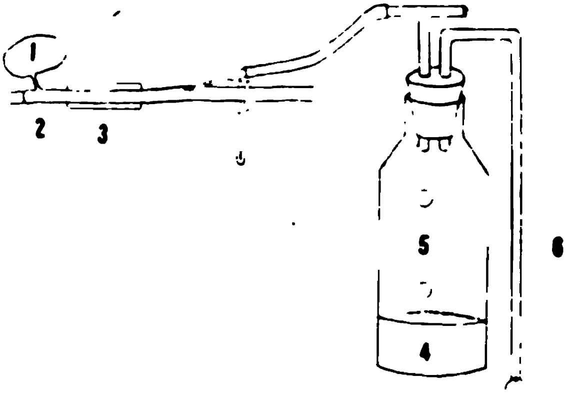


Fig. 4. Oil drop recorder. *1*, Submaxillary gland; *2*, external jugular vein; *3*, volume-flow recorder; *4*, blood; *5*, oil; *6*, siphon; *7*, dropper.

In employing the drop method for quantitative work, difficulties in maintaining a constant size of the drop due to clotting of the blood occur. These are overcome by leading the blood into a bottle containing oil, communicating to the exterior by an overhanging siphon (see fig. 4). The blood as it enters the bottle sinks and displaces the oil. This oil is siphoned off and exerts a small suction on the vein. At the end of the siphon is a specially constructed dropper to insure a uniform drop of oil. The oil emerges at the side of the dropper as shown, instead of the bottom, and runs down the outer surface of a perfect brass sphere. Any slight change in position of the dropper will therefore have no effect on the size of the drop. An additional precaution is necessary and that is to keep the lower surface of the dropper free from clinging air bubbles. The needle which is used for drawing the blood is inserted with the bevel facing downward and is held in that position with slight upward tension on the vein, by the rod of block tin.

The two methods described for testing the accuracy of the instrument are the methods also used in calibrating the instrument in quantitative experiments.

If the first method is used (calibration by injection), the flow of blood from the tissue under study is temporarily shut off and a known amount of solution run into the vein above the instrument, the solution after passing the instrument entering the general circulation.

In the second method (calibration by bleeding), the blood passing the instrument during the period of calibration is lost to the animal.

Calibration by bleeding is the simplest method and the one to be preferred, but the method selected will depend upon the amount of blood flowing from the tissue and the degree to which hemorrhage affects the experiment. The period of calibration, at the most, need not be much over a half-minute, and in such a case as the submaxillary gland the loss of blood is negligible and therefore calibration by bleeding is the method of choice (should the loss of even small quantities of blood be detrimental to the experiment, reinjection of the blood which passes the instrument should be an easy matter). Where the blood flow is freer, as in the case of the kidney, calibration by injection is the method of choice.

If a calibration is to be of service for any length of time we must be sure that the caliber of the vein does not vary. The greatest source of caliber change is drying of the vein. This can be avoided by the use of absorbent cotton moistened in saline which, pulled out into a thin sheet is light enough to offer no impediment to the flow of blood when placed on the vein. The whole of the exposed vein with the exception of the part in the trough is covered and with an occasional moistening of the cotton and an occasional drop of saline in the trough, the vein keeps in perfect condition through prolonged experiments up to death. Taking such precautions I have found the calibration to remain constant for periods of an hour and a half or more.

The automatic volume-flow recorder was developed primarily for studying the volume-flow of blood through the submaxillary gland. It has, however, a wider application. The blood of any of the tissues such as skin, fat, bone or muscle that can be led into a larger vein, can be measured. The instrument is so shaped that it can be inserted into the abdomen. It is therefore possible to measure with it the flow of blood through the adrenal glands, for example. In a few experiments in which the volume-flow of the kidney was measured, the method proved satisfactory. For recording the flow of this organ, the inferior vena cava serves as the reservoir under the emptying-plate. By ligating the cava at the iliac bifurcation, a reservoir of maximum size is obtained. Whether this reservoir is of sufficient size to accommodate the volume-flow of any kidney under any condition has not been determined. The application of the method to various organs and tissues is a problem in itself which has not been thoroughly looked into.

SUMMARY

An automatic and bloodless method of recording the volume-flow of blood is described.

With this method the blood, without coming in contact with any foreign substance, is measured as it flows through a vein on its way to the heart.

The blood to be measured is led into a large vein. This vein, serving as a reservoir, is placed in the trough of the volume-flow recorder.

By means of solenoids and electrical contacts operating a cut-off and an emptying-plate, the vein automatically fills and empties, the blood flowing on to the heart.

The frequency of filling and emptying varies in direct proportion to the volume-flow of blood and is recorded by a signal magnet in circuit with one of the solenoids.

The capacity of the vein lying in the trough may be varied mechanically by raising or lowering the cut-off contact. This adjustment

permits the adaptation of the instrument to different rates of blood-flow.

The method may be used to measure the volume-flow of blood from a number of tissues. Its full application, however, has not yet been worked out.

The advantages of the method are: *a*, by calibration of the vein the procedure is made quantitative without the usual direct measurements of the blood; *b*, it is automatic and therefore requires very little attention; *c*, it is bloodless and therefore does not require the use of anti-coagulants; and *d*, it may be used over long periods of time without affecting the general condition of the animal.

STUDIES ON THE SUBMAXILLARY GLAND

III. Some Factors Controlling the Volume-Flow of Blood[1]

ROBERT GESELL

From the Physiological Laboratory of Washington University School of Medicine, St. Louis

Received for publication October 26, 1918

INTRODUCTORY

The original purpose of this paper was to study in detail certain phases of the electrical deflections of the salivary gland in an attempt to reach an interpretation of the components described in the first paper. For an understanding of the significance of these components an insight into the nature and sequence of the metabolic processes occurring during secretion, recovery and rest is needed and for that reason the metabolism of the gland was studied under varying conditions.

While this work was in progress it assumed a practical as well as a purely theoretical interest in connection with the problem of shock. Though shock may be initiated by a variety of forms of tissue abuse—anaesthesia, cold, fatigue, thirst, etc., along with inflicted injury—the disturbances set up by these forms of tissue abuse might in many instances be of lesser importance were it not for their associated circulatory disturbances. Even after largely removing the initiating causes of shock their detrimental effects on volume-flow may persist and help to sustain the condition[2].

Since normal as well as abused tissues are dependent upon an adequate volume-flow of blood the effects of decreased flow appeared to be most important, the most amenable to study and to treatment. It is a matter of some importance, therefore, to determine the flow which is adequate for normal nutrition.

---

[1] Reported in part before the American Physiological Society, Proceedings in this Journal, 1918, xlv, 545.

[2] See page 469 of paper IV for a fuller discussion

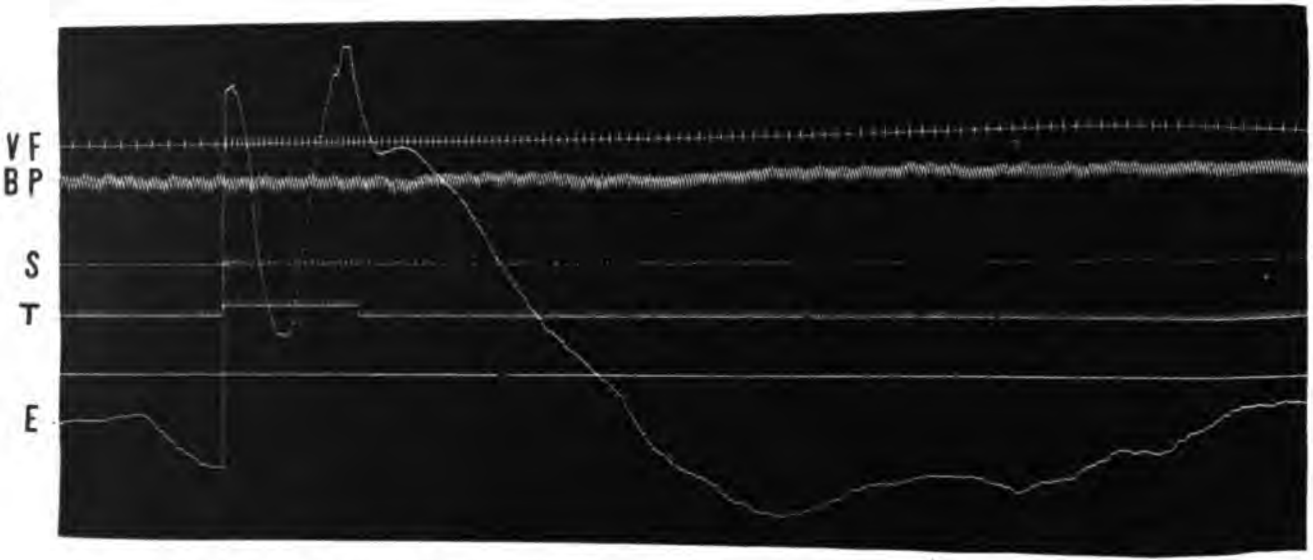


Fig. 1. Record showing parallel fluctuations in secretion, volume-flow of blood and electrical-deflection elicited by prolonged chorda stimulation. *V.F.*, volume-flow of blood; *B.P.*, blood pressure; *S.*, secretion; *T.*, time and stimulation, *E.*, electrical deflections.

The question of determining the minimal flow of blood necessary to maintain the tissues in a normal condition is by no means an easy one for we know that tissues, even though the flow of blood is very low or even absent, may vigorously respond to stimulation. Tissues therefore, evidently possess a factor of safety both in the form of stored energy and in the form of ability to work under unfavorable conditions for a long period of time without sustaining injury, but on the other hand there is the possibility that tissues apparently responding in a normal way may be undergoing a progressive decline. It seemed that this question could be elucidated in part by a study of the mechanism regulating the volume-flow in both active and resting tissue by the methods developed in the study of electrical deflections of the submaxillary gland.

The view that vaso-dilator nerves are of fundamental importance in producing increased blood-flow during tissue activation is accepted by many. Others believe that the action of vasodilator fibers has not been definitely proved and that the increased metabolism always precedes and is the peripheral chemical cause of the increased flow of blood. (Gaskell, Barcroft and Henderson and Loewi and others.)

It was this problem of the mechanism of the control of volume-flow of blood, begun in connection with the observations of the synchronous fluctuation of the electrical deflection, of secretion and of volume-flow of blood accompanying prolonged chorda stimulation that I was studying when the interest in shock developed. (See fig. 1.) It seemed that the two problems, the theoretical and the practical, might be followed simultaneously and it should be mentioned here that the results in this paper will be considered primarily from a theoretical point of view. Their practical application will be considered in the following paper.

THEORETICAL

When the chorda lingual nerve is stimulated for a period of a minute or more and the associated electrical deflection recorded, crests 1, 2 and 3 usually appear. If the secretion and the volume-flow of blood are likewise recorded fluctuations corresponding with crests 2 and 3 occur. (See fig. 1.)

These fluctuations in secretion may be variously explained. They may be due to causes other than the synchronous changes in volume-flow of blood but if volume-flow is a factor it is a question of theoretical and practical interest to know the sequence of events; whether increased volume-flow of blood is primary, accelerating secretion, or whether it is secondary to an accumulation of metabolites accelerating the volume-flow of blood.

Causes of secretory fluctuations during prolonged chorda stimulation. *a.* The fluctuations in secretion may be the result of inhibitory action of the chorda tympani fibers upon the secreting cells; thus Langley (1) found after incomplete paralytic doses of nicotine that salivary secretion produced by chorda stimulation was mainly an after-effect. Dale and Laidlaw (2) likewise noted that if the chorda tympani is stimulated during the rapid secretion occurring at the cessation of the original stimulation, the secretion is again retarded. Applying this observation to more normal conditions, they state that

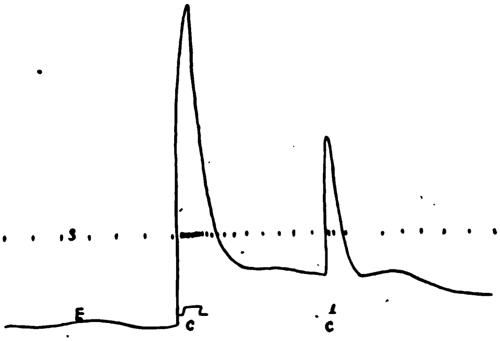


Fig. 2. After-secretion showing effect of short chorda stimulation. *S.*, salivary secretion, *E.*, electrical deflection, *C.*, chorda stimulation.

> there is an indication of double effect of the chorda even before the administration of an alkaloid, in that the rate of secretion falls off towards the end of prolonged stimulation to be accelerated again at the end of stimulation.

In this research these results have been repeatedly confirmed. See the effect of prolonged chorda stimulation on secretory fluctuations in figure 1 followed by an after-secretion. In figure 2 note the effects of short stimulation during the period of after-secretion. The primary increase in rate of salivary flow suggests a contraction of the ducts expressing the contained secretion. The subsequent period of retarded flow suggests a dilatation of the ducts taking up the steady after-secretion. These results bring into question whether in the case of prolonged chorda stimulation the slowing of secretion following the first rapid secretion is an effect of secretory inhibitory fibers or is due to changes in caliber of the salivary ducts.

b. The fluctuations of secretion may also be connected with variation in the amount of water directly at the disposal of the cells for secretion; for Barcroft (3) found it necessary to ignore the first minute of stimulation to obtain a constant relation between the amount of saliva secreted and the amount of water lost by the blood in passing through the gland. The observations of Bunch (4) on volume changes in the gland during chorda stimulation point in the same direction.

c. A possible factor operating to produce secretory fluctuations is the effect which a sudden increase in metabolism itself has upon subsequent metabolism, as demonstrated in the staircase phenomenon in a series of muscular contractions. A concentration of metabolites developed during the period in which the pre-formed saliva is being liberated might have an effect upon further response to prolonged chorda stimulation.

d and *e.* Two other possible explanations of the fluctuation in secretion have to do with the interdependence of volume-flow of blood and metabolism, the particular phase of the problem in which we are now interested.

The second increase in secretion may be due to the second increase in volume-flow of blood, but on the other hand the increase in volume-flow of blood may follow an increase in metabolites resulting from the increased secretion.

To throw light on this question of the control of volume-flow and on the dependence of nutrition on volume-flow certain mechanical, nervous and chemical factors affecting flow of blood were studied.

The effect of arterial compression on the response of the gland to stimulation of the chorda tympani. Interference with the blood-flow to the gland should permit the study of combined effects of ischaemia and of greater concentration of metabolites during chorda stimulation. Though considerable work has been done on this problem (5), (6), (7) and (8), I was interested, during the study of electrical deflections in another phase, namely, the effects of arterial compression on the electrical deflections. Some results then obtained are given here.

Assuming that compression of the artery invariably decreases the volume-flow of blood, we might expect a uniformity of results. For the most part a decrease in the response of the gland to stimulation occurred but striking exceptions were noted. I hesitate to describe these results for the reason that they have not been noted by others and that later when an interpretation of the results was sought by a synchronous record of the volume-flow of blood, they could not be duplicated. They must, therefore, be taken for what they are worth.

Figure 3, *A*, of experiment 46 shows the results of chorda stimulation with and without compression of the artery. Compression in each instance accelerates secretion with an apparent alteration of the processes of secretion. At least this is suggested by the electrical deflection, for the deflection on compression is increased out of all proportion to the acceleration of the secretion.

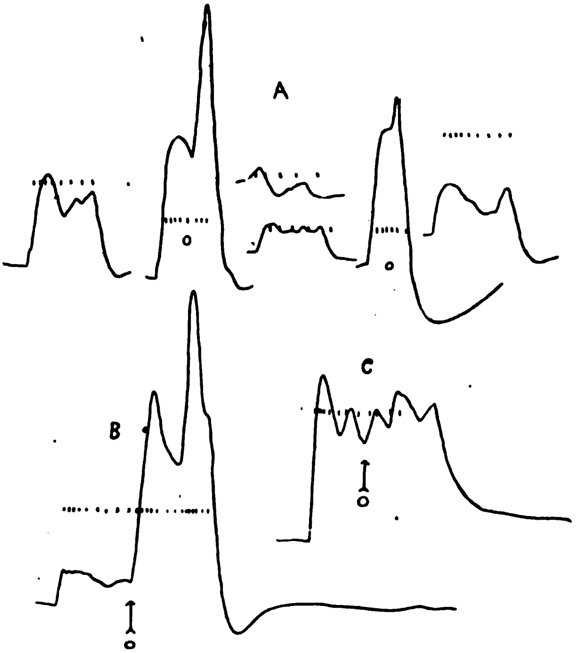


Fig. 3. Records showing effect of arterial obstruction on secretion and electrical deflection with chorda stimulation. The records marked *O* were obtained during obstruction. Figures 3 *A* and *B* are from exp. 46, figure 3 *C* from exp. 47.

Figure 3, *B*, of experiment 46 shows the effect of carotid compression during prolonged chorda stimulation. The acceleration in secretion and the disproportionate increase in the deflection occur again. Another point in this record exciting speculation is that although crests 2 and 3 appear during chorda stimulation before obstruction, they are duplicated by arterial obstruction. In figure 3, *C*, experiment

47, the enhancing effects of arterial compression are small and transitory yet the acceleration of secretion with the duplication of crests 2 and 3 occurs again.

The experiments indicate the possibility of a transitory stimulation by certain grades of ischaemia. The possibility that the response might be due to an effect of central asphyxia transmitted to the gland through the sympathetic fibers occurred to me too late to make the necessary controls.

Observations on the volume-flow of blood during prolonged chorda stimulation without carotid obstruction. While some of the results of arterial compression might suggest that certain grades of asphyxia have an enhancing effect on secretion, the records of volume-flow during chorda stimulation in agreement with most of the results obtained with arterial compression, seem to suggest the reverse. For they show fluctuations in volume-flow running parallel with the fluctuations in secretion and electrical deflections. (See fig. 1).

The interpretation of these results depends upon the exact sequence of changes in secretion and in the volume-flow of blood and upon the extent the two are dependent on each other. An answer to this question might decide the issue between the metabolite and the vasomotor nerve control of volume-flow of blood.

The fluctuations in volume-flow are probably not due to the abstraction of water from the blood, for in the first place the secretion formed is not as great as the fluctuation in the volume-flow. In the second place, identical changes in volume-flow of blood can be elicited by a weak stimulation that fails to produce secretion in the normal gland, and by strong stimulation of the chorda tympani after a paralytic injection of atropin sulphate. The fluctuations in volume-flow of blood, therefore, appear to be vasomotor phenomena, either of nervous or metabolic origin.

a. It is difficult to devise a direct experiment to determine the time relations between vasodilatation and metabolism (secretion) owing to the difficulty in determining the moment of vasodilatation. The latent period of secretion elicited by chorda stimulation as measured by the appearance of saliva is variable but when measured by the electrical deflection the response of the gland is prompt, so that the moment of stimulation marks closely the beginning of secretion. The latent period of dilatation as measured by increased volume-flow of blood is prolonged by the filling of the capillary bed.

b. Atropinization experiments seemed for a time to give direct evidence of independent systems of fibers, vasodilator and secretory, for after apparently paralyzing the secretory function of the chorda tympani fibers, electrical stimulation still produces an increase in volume-flow of blood suggesting the unimportance of metabolites. Barcroft (9), however, finds even after atropinization chorda stimulation still accelerates the gaseous metabolism of the gland and, therefore, the metabolite theory of vasodilatation is not invalidated.

c. Another apparently direct method which has been used to demonstrate the presence of two sets of fibers is the grading of stimulation. Fibers differing so much in function as secretory and vasodilator fibers might well differ in their threshold of stimulation. A number of workers have elicited, with weak stimulation, an increased volume-flow of blood without an accompanying secretion, concluding the presence of both types of fibers in the chorda tympani. I have had no difficulty in duplicating these results, but synchronous records of the electrical deflections suggest that even though the conclusion regarding the presence of the two sets of fibers may be correct, it is not well founded. When a stimulus just below the threshold for visible secretion is employed, a typical deflection, though not as high as the deflection accompanying secretion, is obtained. Though with decreasing strength of stimulation the deflection decreases in amplitude along with the decrease in volume-flow of blood, it still possesses its usual contour. The deflection and the increased volume-flow of blood finally disappear together, therefore, if the two sets of fibers do exist they appear to have the same threshold of stimulation; or, stated in another way, with chorda stimulation we never obtained increased volume-flow of blood without an accompanying increased metabolism.

The validity of this statement depends upon the interpretation of the electrical deflection and it should be stated here that the deflections cannot be accounted for by changes in volume-flow of blood per se.

The direct methods of measuring the latent periods of secretion and vasodilatation, of paralyzing one set of fibers, leaving the other intact, and selective stimulation by grading of stimulation, have failed, therefore, to give conclusive data. A further search for direct methods proving unsuccessful, an endeavor was made to gain the desired information by indirect methods. It was hoped that in this way a mass of indirect evidence might be obtained from which conclusions in favor of one or the other of the two theories of volume-flow control might be reached.

The relation of volume-flow of blood to superbasal metabolism with a constant head of blood pressure. Chorda stimulation produces vasodilatation by inhibition of the intrinsic and central tonus of the vessels. Assuming the tonus to remain constant the volume-flow of blood might roughly vary as the intensity of chorda stimulation irrespective of whether the control is chemical or through vasomotor nerves.

However the results from vago-sympathetic stimulation, as is well known, vary. In the dog secretion is almost invariably accompanied by a decrease in volume-flow of blood, while in the cat it often is associated with an increased flow. Barcroft (10) pointed out that the difference in the results depends upon the balance of the vasoconstrictor effects of the constrictor fibers and the dilator effect of the metabolites liberated in secretion. Since the sympathetic secretion is very much more copious in the cat, the metabolites gain the upper hand. Carlson (11) takes exception to this view. Employing methods of selective stimulation he concludes that the cervical sympathetic of the cat contains vasodilator fibers as well as vasoconstrictor and secretory fibers.

The same question with regard to the relation of volume-flow control to vago-sympathetic stimulation arises in the case of the dog for in a few experiments I have observed that stimulation of the vago-sympathetic, strong enough to produce secretion, has elicited an increased after-flow of blood. (See fig. 4).

Langley (12) found that combined stimulation of the chorda tympani and the vago-sympathetic may elicit secretion equal to the sum of separate stimulations. Von Frey (13) found that the volume-flow of blood with combined stimulation was less than the flow elicited by chorda stimulation though it may be greater than the flow of rest.

By recording simultaneously the volume-flow of blood and the secretion with separate and combined stimulation of the chorda tympani and the vago-sympathetic, I have found that the results of Langley and Von Frey may be combined; that is, that with combined stimulation of the two sets of nerves we may get a summation of secretion with a flow of blood less than that elicited by chorda stimulation alone. (See fig. 4).

It is apparent, then, that the quantitative relation between increased metabolism and increased volume-flow of blood is a variable one depending upon the conditions of the experiment; that the vasoconstric-

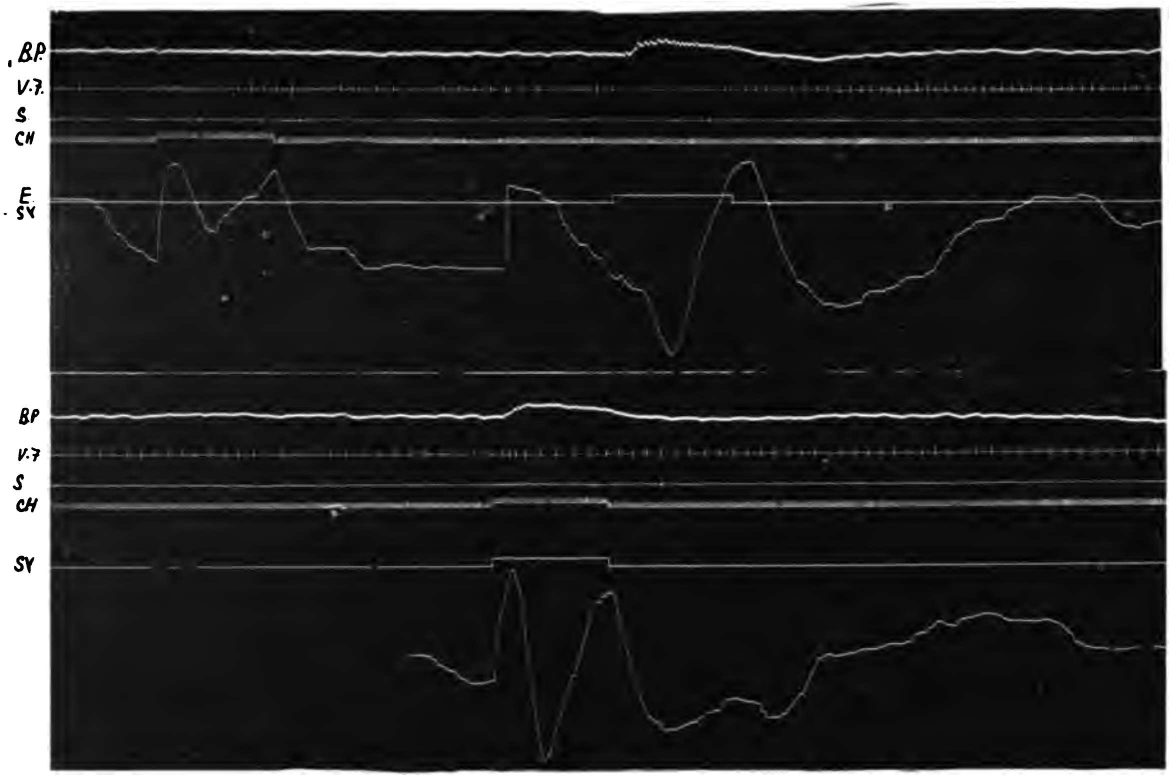


Fig. 4. Three records showing separate and combined effect of chorda and vago-sympathetic stimulation on the secretion, volume-flow of blood and electrical deflections. An increased after-flow of blood following vago-sympathetic stimulation is also shown.

tor tonus and its inhibition is an important factor in controlling the volume-flow. In making a quantitative study of the relation of metabolism to volume-flow of blood, it is best to activate the gland by stimulating the chorda tympani nerve in which there are no or at least few constrictor fibers. Under such circumstances we can assume the potential vasoconstrictor tonus to be constant.

Assuming that metabolites control the volume-flow of blood, it might be fair to expect the flow to bear a direct relation to the amount of metabolism. With chorda stimulation such a relation seems to be more essential for the metabolite mechanism of control than for the nervous and therefore, if found, should add support to the former theory.

In the method used to study this relation it is presumed that the resting or basal metabolism of the gland calls for just enough blood to carry on that metabolism in a normal fashion. Any extra flow of blood produced by activating the gland is called for by the extra or superbasal metabolism. With the general condition of the animal constant, the chorda tympani was stimulated at regular intervals of ten minutes, and one, two, four, six, etc., drops of saliva elicited by varying, but relatively short, periods of stimulation. The salivary secretion was recorded by the drop method and the size of the drop clinging to the dropper at the end of secretion estimated. The volume-flow of blood was recorded with the bloodless method to insure a constant condition of the animal. Subtracting the basal flow of blood for a period of five minutes preceding stimulation from the flow—basal plus superbasal—of the following five minutes initiated by the stimulation, we get the secretory or superbasal flow. Plotting the superbasal flow on the ordinates against the amount of secretion on the abscissas we find the relation of flow to metabolism to be represented by a straight line. (See fig. 5).

When the secretory blood-flow is divided by the amount of secretion we do not get a constant. This interesting feature is shown in the curve. It will be noted that the curve at zero secretion does not end at the intersection of the ordinates and abscissas but rather at a point on the ordinates above the abscissas, in agreement with the observation cited before that even though chorda stimulation may be too weak to elicit secretion yet the electrical deflection accompanying the increased flow of blood indicates that the gland has been activated.

Though not necessarily ruling out the nervous regulation of blood vessels, the linear relation of volume-flow of blood to glandular metabolism is suggestive of a purposive mechanism of volume-flow control. The value of the results in their bearing on the theory of control is to some extent a matter of opinion—the question is whether or not we might expect so fine an adjustment of flow with stimulation of vasomotor fibers entirely independent of metabolism. Would one be justified in expecting, under independent control, an occasional disturbance of adjustment? For this reason the observations were repeated in six animals. In twelve curves plotted the linear relation held.

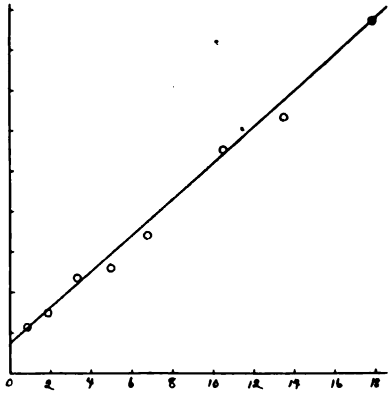


Fig. 5

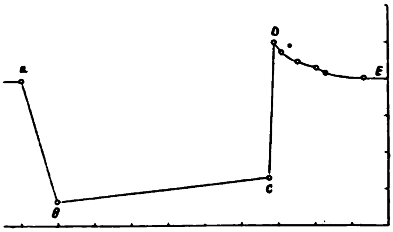


Fig. 6

Fig, 5. Curve of superbasal-flow of blood plotted on ordinates against drops of secretion on the abscissas.

Fig. 6. Effects of arterial obstruction on the basal-flow of blood. The carotid artery was obstructed at *A* and released at *C*. Normal flow at *A*. The blood pressure at *A* and *D* is equal.

The relation of basal flow of blood to basal metabolism. To test the assumption made above, that the basal flow of blood also bears a linear relation to basal metabolism the effect of depriving the gland of its normal flow of blood was studied. I hoped by producing asphyxial conditions of different degrees (time of obstruction) and measuring the after acceleration of blood-flow during recovery when the obstruction is removed, that a quantitative study of the relation of volume-flow to basal metabolism might be made.

The effects of partially obstructing the artery for a few minutes are shown in figure 6 in which the volume-flow of blood is plotted on the ordinates against time on the abscissas. After diminution of the volume-flow to the minimum at *B* there is a steady acceleration up to *C*, where the obstruction is removed. Then follows a flow considerably faster but soon returning to normal.

The effects of obstruction are striking and the method, therefore, gave promise of yielding quantitative data. But a difficulty in interpretation became apparent, in the form of an additional variable,—the effect of tension on the tone of blood vessels—making a quantitative study of the effect of asphyxia difficult. The effect of tension on blood vessels was pointed out by Bayliss (14). The same effect of tension on the tone of the auricle of the turtle was also noted by myself (15). Increasing the filling tension of an automatically beating auricle suddenly increases the auricular volume, followed by a development of tonus as shown by a slowly decreasing volume. These volume curves resemble somewhat the curve of volume-flow of blood in figure 6. This method for determining the relation of basal-flow to basal metabolism, therefore, is not quantitative, for the question naturally arises, how much is the acceleration of blood flow from *B* to *C* and at *D* due to the diminution of tone from concentration of metabolites or to decreased tension; and how much of the decreased flow from *D* to *E* is due to a washing out of metabolites or to increased tension.

The relation of superbasal flow of blood to superbasal metabolism with decreasing blood pressure. The observations on this point have a double interest; one, in the relation to the mechanism of volume-flow control, the other in the relation to the actual amount of superbasal-flow for a given amount of work at progressively lowered blood pressures. Here we are interested mainly in the former question.

With all other conditions of the animal kept as constant as possible, equal amounts of secretion were elicited by chorda stimulation at progressively lowered blood pressures secured by haemorrhage. It was hoped that by plotting the curves of secretion and the curves of volume-flow the mechanism of volume-flow might be suggested. If vasodilatation and metabolism vary independently of each other, the amplitude of the volume flow curve on account of the diminution in the driving pressure, would be diminished but the time relations of the two curves would remain the same. If metabolites have an effect, the amplitude of the volume-flow curve may likewise be reduced, the accelerated volume-flow of blood, however, would be prolonged until the metabolites were disposed of, counteracting the decreasing blood pressure and tending to keep the superbasal-flow constant.

A series of curves of secretion and volume-flow of blood are shown in figure 7. In each instance approximately eight drops of saliva were elicited. The blood pressures at which stimulation occurred are designated. The curves beginning above the base line are curves of volume-flow of blood, the horizontal portion preceding chorda stimulation representing the basal-flow of blood. The triangular portion above the basal-flow represents the superbasal-flow (for data see table 1). With decreasing blood pressure the area of the triangle diminishes and at about 34 mm. Hg. the flow of blood is no longer increased by chorda stimulation. At lower pressures the flow is actually decreased.

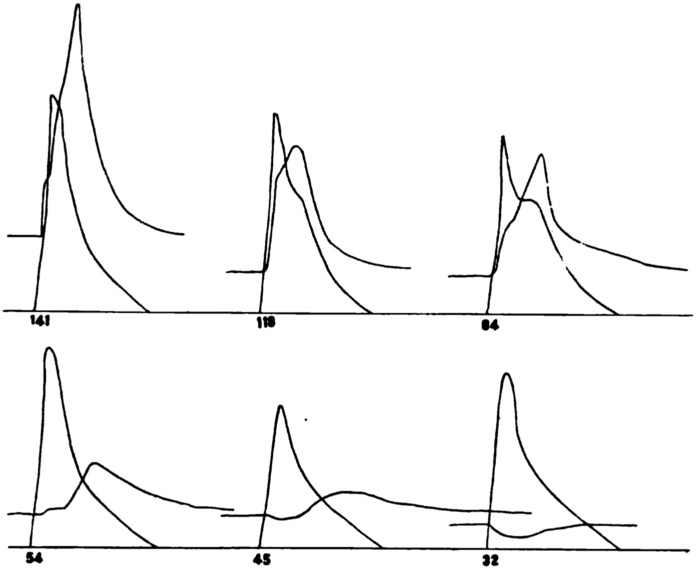


Fig. 7. Curves showing both basal and superbasal-flow of blood at varying designated blood pressures.

The practical importance of these results in conditions of lowered blood pressure is obvious. In relation to the theories of volume-flow control the fact that the superbasal-flow decreases so rapidly and that the effect of decreased blood pressure is not better counteracted seems to speak against metabolite control—but the very great importance of pressure not only as a driving force, but also as a stretching force, the increased construction of central origin from haemorrhage and the possibility of passive constriction with decreasing head of pressure, might overrule this objection and will be discussed later. The fact that the decreased pressure is somewhat counteracted, as evidenced by the prolongation of the after-flow, indicates that the volume-flow of blood is in part, at least, dependent on metabolites.

The relation of basal-flow of blood to mean blood pressure. There seems to be little quantitative data on record on the relation of basal-flow of blood to decreased blood pressure produced by haemorrhage. If driving pressure is the only variable, the volume-flow of blood would steadily decrease and when plotted on the ordinates against blood pressure on the abscissas would be represented by a straight line. If a deflection of this curve occurs some other variable has come into play.

TABLE 1

OBSERVATION	BLOOD PRESSURE IN MM Hg	BASAL BLOOD FLOW IN DROPS PER MINUTE	SECRETORY BLOOD FLOW + SECRETION DIVIDED BY SECRETION
6	141	39.5	9.5
11	129	27.7	8.6
14	115	21.0	5.8
16	84	20.0	5.8
20	60	19.0	6.8
21	54	18.0	3.7
22	49	17.2	3.7
23	45	18.0	3.3
24	41	17.4	2.1
25	34	17.2	1.0
26	34	16.6	0.0
28	32	16.0	Lower than basal flow
29	29	13.7	
30	28	11.1	

These experiments were performed to find how the volume-flow of blood is affected by a fall in pressure, in other words whether the dilating effect of metabolites might be demonstrated by a bending of the curve, and by this bending the volume-flow of blood necessary for normal tissue metabolism might be determined. It seemed that the volume-flow might decrease with the fall in pressure up to a point where the flow of blood was no longer sufficient to carry off the metabolites, permitting a sufficient concentration to affect vasodilatation, or an upward bend of the curve. This would counteract the decreasing head of pressure and tend to keep a constant volume-flow acting as a protective mechanism to maintain adequate nutrition. The point at which the rapid decrease in volume-flow of blood is checked by the hypothetical concentration of metabolites would indicate approximately the volume-flow essential to the tissues.

The basal-flow of blood was measured by the drop method and plotted on the ordinates against mean blood pressure on the abscissas. (See fig. 8.)

Since the flow of blood is not represented by a straight line, according to the law of Poiseuille, driving pressure cannot be the only variable. One or more additional factors equally as important enter into play dividing the curve into three distinct parts—the first, in which the flow decreases decidedly faster than the fall in blood pressure; the second, or the plateau, in which the rapid decrease is checked; the third, in which the flow again decreases faster than the fall in pressure.

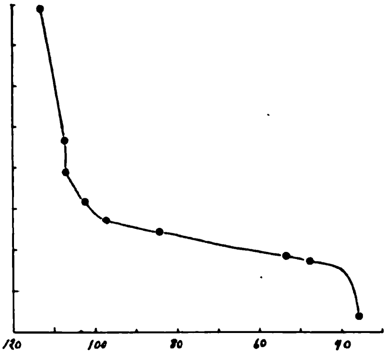


Fig. 8. Curve of basal flow of blood obtained in a haemorrhage experiment. Flow is plotted on the ordinates against blood pressure on the abscissas.

The change in flow during the second period is variable. The flow may decrease but always much more slowly than the decrease in pressure, it may remain constant or in some instances actually increase. Despite the agreement of the curve with the working hypothesis, a checking up of the factors controlling volume-flow seemed called for.

The three main factors determining the rate of flow are driving pressure, viscosity of the blood and caliber of the vessels.

Viscosity. The viscosity of the blood depends primarily upon the number of corpuscles. In haemorrhage there is a progressive dilution of blood from the outset. The rapid fall in volume-flow cannot be attributed to, but actually is counteracted by dilution of the blood. That the sharp bend in the curve cannot be attributed to the counteracting influence of dilution is also certain. In some animals in which the blood dilutes rapidly the decreased viscosity may be a factor of some importance, but in these experiments on the dog in which the percentage of corpuscles was followed, the slow and even dilution of the blood rules out viscosity as an important factor. (See fig. 4, exp. 87, page 485.)

Injecting 7 per cent gum acacia to raise the pressure after extensive haemorrhage produces a volume-flow of blood greater for the same head of pressure during the haemorrhage. This may be explained either by a dilution of the blood decreasing the viscosity or by a dilatation of the vessels permitting a greater flow. The point of interest is that the contour of the injection curve of flow duplicates the haemorrhage curve. (See fig. 7, exp. 49, page 490.)

The converse also holds. When the blood pressure is reduced by tissue abuse it is accompanied by a concentration of the blood yet the curve of volume-flow is essentially the same as in haemorrhage. Changing viscosity of the blood, therefore, affects but little the contour of the volume-flow curve. (See fig. 7, exp. 48 and 40, page 490.)

Caliber of the vessels. The breaks in the curve, therefore, are due mainly, to a change in caliber of the vessels. In part 1 there is a constriction; in part 2 a checking of this constriction, in part 3 a second constriction.

The gradient of the first part of the curve and the point at which it is checked varies in different animals and apparently depends upon a number of factors—the tonus of the blood vessels at the beginning of haemorrhage and the relative importance of active and passive constriction. In haemorrhage active constriction is a well-known reaction. One frequently meets with animals reacting to a large haemorrhage with a rise instead of a fall in blood pressure, the volume-flow of blood markedly decreasing.

Every effort was made to avoid complicating the curve with the effects of cold and of inflammation. In the first place, the gland is dissected carefully. Frequently, when the veins are conveniently placed, the dissection requires no manipulation of the gland whatever. The gland is re-covered by the overlying skin and a protecting layer of gauze. That a more or less permanent change in the vessels, such as is associated with inflammation, does not occur is shown in the re-injection experiments, where the volume-flow curve with decreasing pressure is duplicated on increasing the pressure by gum acacia injection. (See fig. 6, page 449, and fig. 7, exp. 49, page 490.)

The decrease in the volume-flow of blood in the first part of the curve during haemorrhage with a rise in pressure can therefore be safely attributed to active vasoconstriction of central origin. This active constriction may continue with the fall in blood pressure. The bend in the curve initiating the plateau may mark the point where the vasomotor center is losing its tonus. It may also mark the point where the concentration of metabolites becomes such that its peripheral dilating effect counteracts the constriction and fall in blood pressure.

The effect of vago-sympathetic section on the curve of volume-flow. By measuring the volume-flow of blood from both glands with the vago-sympathetic intact on one side and cut on the other we have apparently a simple method for determining the relative importance of vasomotor changes of central or peripheral origin in different parts of the volume-flow curve. But the effects of section of one nerve proved to be a problem in itself and were followed out only in part at this point. The eight experiments performed, however, were of some value in furnishing material for the study of volume-flow control and are therefore described.

The flow was measured by the drop method; the animal was bled continuously up to death through the two glands. At the beginning of the experiment the vago-sympathetic on the right side was cut.

A few experiments showed that the effects of section of the vago-sympathetic were not always limited to one side alone but that dilatation of varying degree occurred on the contra-lateral side as well. On section of the nerve, the volume-flow increases rapidly, reaching its maximum in about a minute and then gradually decreasing. The normal tone on the side of the cut nerve is usually reached in about five to seven minutes. These points are brought out in figure 9 and table 2. In experiment 79 the effect of section of the vago-sympathetic is apparently limited to the right side alone. The slight increase in volume-flow of the left side can be attributed to the increased blood-pressure. On the right side the maximum flow is 333 per cent of initial flow, but in five minutes when the initial blood pressure is reached again the flow is 54 drops per minute, or the initial rate The tonus of the vessels of the left gland in the meantime has increased beyond the normal, the flow decreasing from 36 to 26.6 drops per minute.

In experiment 78 the effect of nerve section is definitely bilateral, for the blood pressure remains constant, yet the flow on the left side is increased. In experiments 77 and 80 (no record shown) the contra-lateral dilatation is very marked.

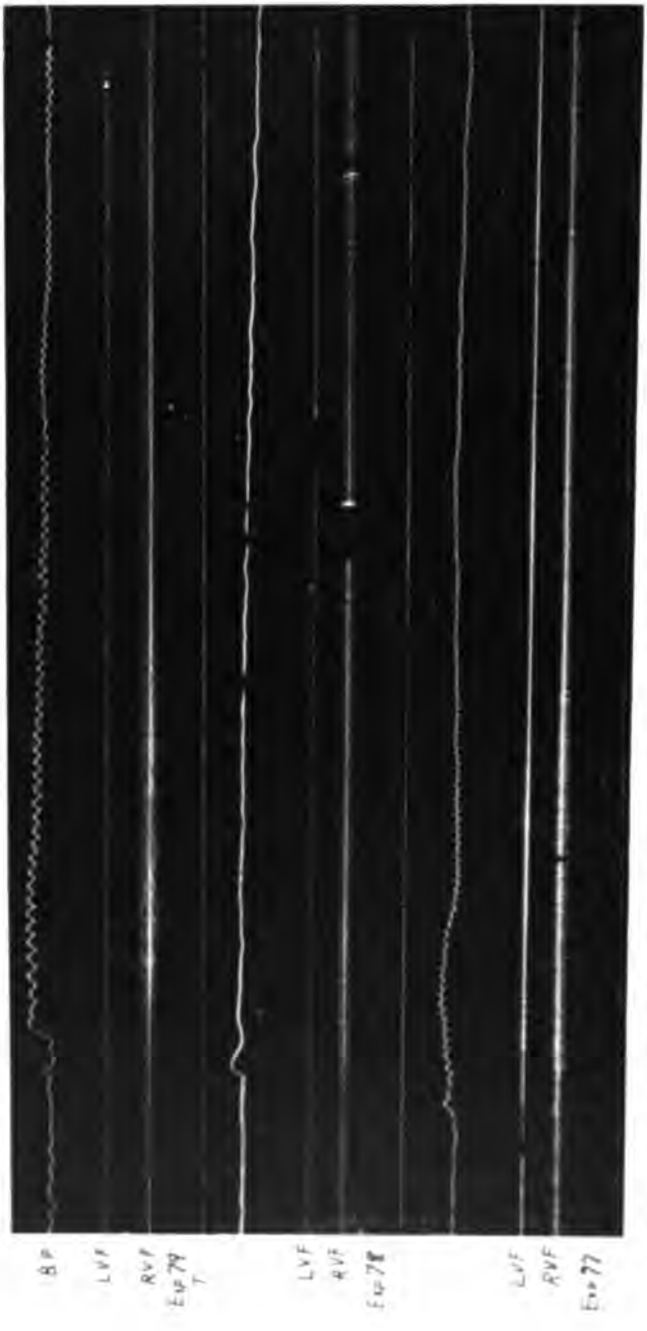


Fig. 9. Records showing homo- and contralateral effects of vago-section on the volume-flow of blood through the submaxillary glands.

If we examine the curves of volume-flow of blood from experiments 77 and 80 we find that the contour is very similar to the contour of curves obtained with the vagi intact, and it is surprising to find the curves of flow of the two sides so nearly alike as shown in the curves from experiment 77. In experiment 80 where the initial flow of the two glands (right 66.6 and left 98.4 drops per minute) varied considerably—right vago-section increased the flow of the two glands to 128 and 130 respectively. On bleeding, the flow from both sides remained so nearly alike at most pressures that only one curve was plotted. (See table 2.) Apparently the function of the vago-sympathetic in this experiment is thrown out more or less completely on both sides, so that both curves may possibly be looked upon as unaffected by central increased tonus working through the vagus nerves. Though in these experiments we get no decreased volume-flow with an elevated pressure or stationary pressure, except where the vessels

TABLE 2

EXPERIMENT 79			EXPERIMENT 78			EXPERIMENT 77			EXPERIMENT 80		
B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.	B. P. in mm. Hg	Rt. V. F. in drops per min.	L. V. F. in drops per min.
108	54.0	36.0	112	29.0	27.0	116	85.0	126.0	110	66.6	98.4
Cut	Rt.	Vagus	Cut	Rt.	Vagus	Cut	Rt.	Vagus	Cut	Rt.	Vagus
121	180.0	41.2	116	90.0	33.0	117	195.0	198.0	105	128.0	130.0
110	54.0	26.6	114	63.5	28.5	121	159.0	152.0	102	110.0	110.0
104	54.0	31.0	107	40.8	24.5	116	105.0	112.0	102	90.0	90.0
100	63.0	33.0	93	25.5	16.3	110	85.0	96.0	100	78.0	79.0
97	61.0	32.0	96	26.3	19.6	105	80.0	90.0	94	56.6	56.6
93	58.4	31.0	87	34.5	23.5	100	73.0	70.0	78	48.0	45.0
92	58.0	31.0	84	36.0	23.0	95	72.0	68.0	72	44.0	43.7
82	55.6	28.6	74	30.5	21.0	90	62.0	50.0	64	38.6	38.4
76	48.5	27.7	63	27.5	22.0	84	46.0	36.0	57	32.4	34.3
71	44.0	25.0	60	22.9	19.6	78	38.0	30.0	50	26.8	28.4
64	37.0	24.0	50	18.8	18.3	70	36.0	26.0	34	18.5	21.4
60	32.5	22.0	41	19.0	18.3	62	27.0	14.0	20	15.0	16.0
55	30.3	21.4	30	38.0	14.0	50	23.0	13.0			
40	26.2	16.0				43	21.0	12.+			
24	29.0	22.0				37	21.5	13.3			
21	35.0	13.4				27	19.2	11.4			
						25	17.6	10.0			
						18	7.1	5.8			

are gaining lost tone, curves 77 and 80 show that a definite vasoconstriction is taking place. This constriction may be passive and due to a decreased stretching head of pressure. Owing to the conflicting data on the action of adrenin in haemorrhage the effect of this hormone cannot be used to explain the results (16). The curves show, at least provided no constriction occurs through the chorda tympani, that a fall in blood pressure when unaccompanied by an active central constriction has serious effects on the volume-flow of blood out of all proportion to the fall in pressure.

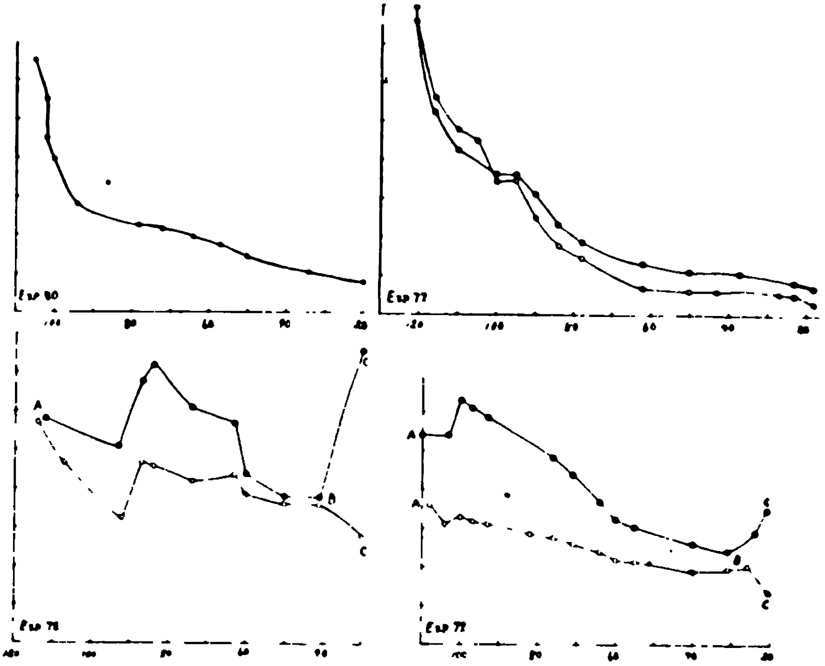


Fig. 10. Curves of basal-flow of blood from both submaxillary glands showing the effect of section of the vago-sympathetic on the right side.

The curves of flow of experiments 78 and 79 show similar irregularities which are also present though not as well marked in the remaining experiments on section of the vago-sympathetic. These irregularities have not been explained but the failure of the volume-flow to decrease in the usual way with decreasing head of pressure suggests some peripheral chemical effect such as might result from disturbed metabolism following vago-section or some central effect working through the chorda tympani, an effect which was not ruled out.

In experiments 78 and 79 the effects of vago-section were mainly homolateral yet the configuration of the curves from *A* to *B* in each pair is very similar. From *B* to *C*, however, there is a marked divergence—a rapid increased volume-flow on the side with the nerve cut though the pressure is falling and a sudden diminution in flow on the side with the innervation intact, suggesting a peripheral chemical dilatation in one gland and an overcoming of this by constriction of central origin in the other.

The relation of alkaline reserve to volume-flow of blood. This divergence of the two curves brings up the question of the nature of the metabolite producing the reaction. The liberation of powerful dilating substances such as have been studied by Dale and Laidlaw (17), Dale and Richards (18) and Hunt (19) offers the possibility of an effective mechanism of dilatation during tissue activation. We must look to other substances, however, to explain this double reaction.

We know that in conditions of lowered blood pressure, particularly when the respiratory center fails to respond, an increase in hydrogen ion concentration develops as a result of the decreased carbon dioxide capacity of the blood and the increase in concentration of carbon dioxide. And we know the central constricting and peripheral dilating effects of carbon dioxide. Mathison (20) states that there appears to be no reason for regarding the respiratory center as a particular mechanism alone possessing the property of responding to small changes in CO_2 tension of the blood. Hooker, Wilson and Connet (21) found that chemicals which excite or depress the respiratory center have the same effect upon the vasomotor center. The absence of a rise of pressure from the administration of carbon dioxide after decerebration was shown by Kaja and Starling (22) and by Henderson (23). Hooker (24) demonstrated the peripheral dilating effect of CO_2 in the perfused pithed frog and also (25) found CO_2 to have a specific effect, due to other than its acid properties, on the respiratory center of the dog. These observations have more recently been confirmed by Scott (26).

The dilating effects of acid injection can be demonstrated when the vago-sympathetic is cut as is shown in figure 11. The injection presumably increases the hydrogen ion concentration as is indicated by the increased pulmonary ventilation. When the vagi are intact, however, the results obtained are variable. Sometimes the flow was increased, unaffected or decreased. The decreased flow presumably is a result of constriction of central origin as is suggested by an accompanying rise of pressure.

Relation of volume-flow to head of pressure in perfused dead organs. The first segment of the curve of volume-flow of blood in the haemorrhage experiments may represent combined active and passive constriction, the second segment may represent a cessation of both, giving way to a dilatation of central and peripheral origin. It seemed to the point, therefore, to determine the relative importance of physiological reactions and purely mechanical factors.

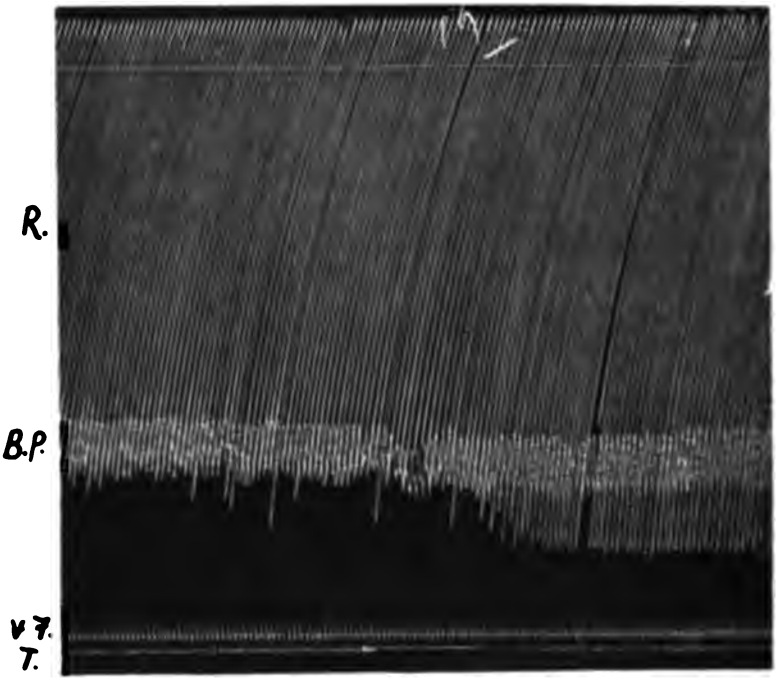


Fig. 11. Record showing the effect of acid injection on respiration and on the volume-flow of blood through the submaxillary gland with the vago-sympathetic cut.

Exceptions to Poiseuille laws have been noted by Denning and Watson (28) and others; e.g., Denning and Watson find that the flow of fluids through inextensible tubes, when plotted on the ordinates against head of pressure on the abscissas, is not represented by a straight line but by a curve with the convexity down. The extent to which this bending occurs increases with the decrease in caliber of the tubes and with an increase in suspended solid particles in the fluid. Such exceptions along with the possibility of a passive constriction of blood vessels accompanying a fall in blood pressure suggested the advisability of artificial perfusion of dead tissue. Kidneys of the dog and cat, excised from one to three days previously, were perfused at room temperature with ox-blood diluted with 7 per cent gum acacia in 0.9 per cent NaCl and also with gum acacia mixture alone. Plotting the volume-flow on the ordinates against driving pressure on the abscissas (fig. 12) we get curves showing a deviation from the straight line. In some instances the deviation is relatively slight, but frequently results shown in figure 12 are obtained. Oedema of the organs was prevented by the employment of acacia. But to rule out this factor completely successive curves of volume-flow were plotted, first with a rising pressure and next with falling pressure. The curves are similar and bear a resemblance to the curves obtained in haemorrhage in the living animal. There are, however, two important differences. The initial decrease in flow is not as rapid as in haemorrhage and the plateau near its beginning points low, that is to the intersection of the ordinates and abscissas at zero. The curve suggests that as driving pressure increases, the vessels stretch which produces an increase in the effectiveness of the driving pressure obtaining. That most of this stretching occurs only after a relatively high pressure (in fig. 12, about 90 mm. Hg.) is reached, is a factor of considerable practical importance in the treatment of lowered blood pressure from haemorrhage and tissue abuse.

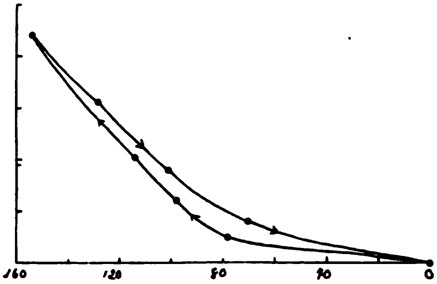


Fig. 12. Perfusion flow of gum acacia through a dead kidney plotted on the ordinates against the head of pressure on the abscissas. The lower curve was obtained by increasing and the upper by decreasing the head of pressure.

That this stretching of vessels in dead organs actually occurs was shown by perfusion with a suspension of blood corpuscles. The percentage of corpuscles of the "venous blood," and it might be stated that the venous flow was slow, fluctuated with the head of pressure. (See table 3.) Apparently the lower perfusion pressures are not sufficient to stretch the vessels or deform the corpuscles sufficiently to permit their free passage and filtration of the blood occurs.

The finding of Denning and Watson (29) that suspended particles increase the deflection of the curve of flow was confirmed by comparing curves of flow obtained with 7 per cent gum acacia suspension alone and a mixture of blood and gum acacia.

It is of considerable interest to know how much the corpuscular factor determines volume-flow in living tissue. If the high peripheral red count noted by Cannon, Fraser and Hooper (30) represents the count of arteriole blood it might indicate a constriction sufficient to block the passage of red cells as they suggested. That such constriction may occur was also pointed out by Cohnstein and Zuntz (31). The lower red counts of venous blood coming from a constricted area, noted by Sherrington and Copeman (32) would also indicate that a constriction may produce a partial filtration of the blood.

TABLE 3

OBSERVATION	BLOOD PRESSURE	RATE OF PERFUSION	PER CENT OF RED CELL IN "VENOUS BLOOD"
1	62	4.6	9.4
2	120	13.8	14.0
3	160	19.5	16.4
4	118	5.4	11.1
5	90	2.6	8.7

The effect of acid injection on the height of the plateau of the curve of volume-flow. From the experiments on perfusion of dead organs it is apparent that purely mechanical factors independent of physiological reactions account in part for the contour of the volume-flow curve obtained from the living animal. Two differences in the curves, however, were noted, one, the lesser deflection in the first segment and the other, the pointing of the plateau to zero on the abscissas. In the living tissue the plateau points well beyond this point, showing definitely that in haemorrhage in addition to the checking of constriction, we have a vascular dilatation accounting for the second deflection of the curve.

Pilcher and Sollmann (33) observed that the vasoconstriction in the early part of haemorrhage merged at a mean blood pressure of 90 to 100 mm. Hg. into a period of dilatation of central origin. The question in which we are interested here is whether this dilatation may be augmented by peripheral chemical dilatation from concentration of metabolites.

It is fully realized that substances other than carbon dioxide may act as chemical regulators of volume-flow, yet the results of experiment 77 suggested experiments along this line. It was hoped that by decreasing the CO_2 capacity of the blood by acid injection the effectiveness of the CO_2 added to the blood as it passes through the tissues would be increased and be demonstrated by dilatation. If this occurs a reduction of alkaline reserve prior to bleeding should elevate the plateau. Variable results were obtained and were attributed to the fact that a reduction in alkaline reserve may increase the effectiveness of CO_2 stimulation of the vasoconstrictor center as well the peripheral mechanism.

The relation of alkaline reserve to superbasal-flow of blood. In the case of superbasal-flow, however, the effects of lowered alkaline reserve might be studied to better advantage for the reason that conditions are more local, the accessions of CO_2 to the blood passing through an activated gland being greater than the accession of CO_2 to the blood passing through the resting vasoconstrictor center. In some experiments acid injection did increase the superbasal-flow. Though a uniform demonstration would be more convincing yet I feel that the greater hydrogen ion concentration established in blood poor in reserve alkalinity as it passes through an activated tissue might be a factor in securing adequate flow and protecting the tissue against still greater acid reactions.

The relation of alkaline reserve to volume-flow is a perplexing one. The double effect of increased hydrogen ion concentration is easily demonstrated. But the question is, can changes in hydrogen ion concentration be looked upon as a normal mechanism of volume-flow control and does the factor enter into play in conditions of lowered blood pressure preceding the marked reaction just prior to death? The problem seems to call for a quantitative study of the threshold and of the difference in threshold of stimulation for the central and peripheral mechanisms.

SUMMARY

This work represents an attempt to obtain data for the interpretation of electrical deflections in the salivary gland by determining the factors controlling the volume-flow of blood and the effects of decreased flow on metabolism.

The experiments were performed on the submaxillary gland of the dog.

By measuring the volume-flow of blood during prolonged chorda-stimulation it was found that the synchronous fluctuations in secretion and electrical deflection were accompanied by parallel changes in volume-flow of blood.

These observations raised the question of the interdependence of volume-flow of blood and tissue metabolism,—are changes in the flow primary, causing fluctuations in secretion or does increased secretion call forth the augmented flow of blood?

The possibility of the secretory fluctuations being an inhibitory phenomenon is discussed.

That the changes in volume-flow of blood exceed the variation in secretion is pointed out; also that fluctuations in volume-flow of blood with chorda stimulation may occur in the absence of visible secretion.

The change in volume-flow of blood with prolonged chorda stimulation is, therefore, a vasomotor phenomenon—either of metabolite or of vasomotor nerve origin.

The measurement of the latent period of secretion and of vasodilatation, the selective paralyzing of the secretory fibers with atropin and the selective stimulation by grading of intensity failed to give conclusive evidence of two independent sets of fibers—vasomotor and secretory.

For that reason the problem was approached by a number of indirect methods which by cumulative evidence might suggest the mechanism of volume-flow control.

Methods were chosen which would yield data of theoretical interest and also of practical value in connection with conditions of lowered blood pressure.

The observations of Barcroft and of Carlson of increased flow of blood elicited by sympathetic stimulation in the cat were confirmed in a few instances in the dog.

Combined stimulation of the chorda tympani and vago-sympathetic may elicit a summation of secretion accompanied by a flow of blood less than that obtaining during chorda stimulation alone.

Since increased volume-flow of blood can occur only through inhibition of the constrictor tonus, a constant potential tonus is required to study the relation of metabolism to volume-flow of blood.

This relation was studied by eliciting progressively increasing amounts of saliva by chorda stimulation during a constant head of pressure and measuring the extra flow of blood.

By plotting the superbasal-flow of blood on the ordinates against metabolism on the abscissas we get a straight line. This suggests a purposive mechanism of volume-flow control though not disproving the theory of nerve control. The apparently perfect adjustment of flow to metabolism might point more strongly to metabolite control.

By studying the time relation of superbasal flow to superbasal metabolism at various heads of pressure, it appears that some of the increased flow of blood is not accounted for by the action of dilator nerves.

The failure of metabolites to counteract more fully the effects of falling pressure may be attributed to the fact that a greater constrictor tonus must be inhibited and that the head of pressure obtaining is insufficient to take advantage of the inhibition produced.

By recording the flow of blood through a resting gland with a decreasing head of pressure suggestions on the relation of basal flow to basal metabolism were obtained.

The sudden decrease in flow giving way to a nearly constant or even accelerated flow with a further fall in pressure supported the working hypothesis—that the flow of blood might decrease to a point permitting a concentration of metabolites in the tissues and tissue blood sufficient to produce a dilatation, thereby tending to maintain a constant flow of blood despite a further fall in pressure.

The importance, however, of a decrease in central constrictor-tonus is evident.

To determine the relative effects of failure of the vasomotor center and the concentration of metabolites curves of flow from glands with the vago-sympathetic intact and severed were compared.

According to the law of Poiseuille, if the factors of caliber and viscosity remain constant the curve of flow plotted against head of pressure is represented by a straight line.

Deflections of this curve from the straight line in the course of haemorrhage must therefore be due to changes either in caliber or in viscosity.

The factor of viscosity has relatively little effect on the general contour of the curve but rather on its position.

The large deflections are, therefore, mainly a function of changes in caliber.

Since in the early stages of haemorrhage there may be a rise of pressure accompanied by a marked decrease in volume-flow the steepness of the first segment of the curve is largely attributable to a vasoconstriction of central origin.

But after vago-section the initial fall in pressure is likewise accompanied by the greatest decrease in volume-flow, suggesting that with the vagus intact both passive constriction and active constriction of central origin occur.

The gradients of the second as well as the first segment of curves with vagi intact and severed are somewhat comparable. The third segments, however, show a marked divergence.

The fact that at low pressures a further decrease in pressure is accompanied on the intact side by a suddenly decreased, and on the severed side by a suddenly increased flow, suggests the possibility of a central and peripheral action of increased concentration of CO_2.

As a control involving only mechanical factors the volume-flow through dead organs with varying heads of pressure was determined.

The presence of the first deflection of the curve shows the occurrence of passive constriction and the importance of a high stretching head of pressure.

The pointing of the plateau to zero on the abscissas, which is not the case in living tissue, shows that in haemorrhage after active and passive constriction have ceased dilatation begins.

The relative importance of failure of the vasomotor center and of concentration of metabolites in elevating the plateau is difficult to determine.

An attempt to raise the plateau by a reduction of the alkaline reserve previous to a lowering of the head of pressure proved unsuccessful, but that the superbasal-flow of blood may be augmented by increasing the effectiveness of CO_2 liberated in metabolism is suggested.

As to the existence of vasodilator nerves, the question which initiated this research, nothing definite can be said.

We have no proof that such nerves do not exist, neither have we proof that metabolites cannot adequately control the volume-flow of blood.

All that can be said at present is that if dilator nerves do control the flow of blood this flow may be augmented still more by an accumulation of metabolites. Many of the observations might apply to both theories, some, however, seem to point more strongly to the theory of metabolite control.

BIBLIOGRAPHY

(1) LANGLEY: Journ. Physiol., 1890, xi, 147.
(2) DALE AND LAIDLAW: Journ. Physiol., 1911, xliii, 196.
(3) BARCROFT: Journ. Physiol., 1900, xxv, 479.
(4) BUNCH: Journ. Physiol., 1900, xxvi, 1.
(5) HEIDENHAIN: Pflüger's Arch., 1878, xvii, 1.
(6) LANGLEY AND FLETCHER: Phil. Trans., cixxx, 109.
(7) CARLSON, GREER AND RECHT: This Journal, 1907, xx, 180.
(8) CARLSON AND McCLEAN: This Journal, 1908, xx, 457.
(9) BARCROFT: Respiratory function of the blood.
(10) BARCROFT: Loc. cit.
(11) CARLSON: This Journal, 1907, xix, 408.
(12) LANGLEY: Journ. Physiol., 1878, l, 96.
(13) VON FREY: Arbeiten aus d. Physiol. Anstalt zu Leipzig, 1877, xi, 89.
(14) BAYLISS: Journ. Physiol., 1902, xxviii, 220.
(15) GESELL: This Journal, 1916, xxxix, 239.
(16) HOSKINS AND WHEELON: This Journal, 1914, xxxiv, 172.
(17) DALE AND LAIDLAW: Journ. Physiol., 1910, xli, 318; 1911, xliii, 182.
(18) DALE AND RICHARDS: Journ. Physiol., 1918, lii, 110.
(19) HUNT: This Journal, 1918, xlv, 197.
(20) MATHISON: Journ. Physiol., 1911, xlii, 283.
(21) HOOKER, WILSON AND CONNET: This Journal, 1917, xliii, 351.
(22) KAYA AND STARLING: Journ. Physiol., 1909, xxxix, 346.
(23) HENDERSON AND HARVEY: This Journal, 1918, xlvi, 533.
(24) HOOKER: This Journal, 1911, xxviii, 361.
(25) HOOKER, WILSON AND CONNET: This Journal, 1917, xliii, 351.
(26) SCOTT: This Journal, 1918, xlvii, 43.
(27) BAYLISS: Brit. Med. Journ., 1918.
(28) DENNING AND WATSON: Proc. Roy. Soc., 1906, 78B, 328.
(29) DENNING AND WATSON: Loc. cit.
(30) CANNON, FRASER AND HOOKER: Journ. Amer. Med. Assoc., 1918, lxx, 526.
(31) COHNSTEIN AND ZUNTZ: Arch. f. d. gesammt. Physiol,, 1888, xlii, 326.
(32) SHERRINGTON AND COPEMAN: Journ. Physiol., 1893, 52.
(33) PILCHER AND SOLLMANN: This Journal, 1914, xxv, 59.

STUDIES ON THE SUBMAXILLARY GLAND

IV. A Comparison of the Effects of Hemorrhage and of Tissue-Abuse in Relation to Secondary Shock[1]

ROBERT GESELL

From the Physiological Laboratory of the Washington University School of Medicine, St. Louis

Received for publication November 1, 1918

This research deals with circulatory and nutritional disturbances resulting from hemorrhage, from tissue-abuse and from acid injection. It is in part an application of the results outlined in the preceding paper as well as a presentation of additional data.

The term shock has been applied to a variety of conditions of lowered blood pressure regardless of whether the condition is attributable to a very sudden disturbance resembling, for example, the immediate inhibition of reflexes following transection of the cord, or associated with a relatively slow and progressive nutritional disturbance accompanying a progressive fall of blood pressure. Attempts have been made to differentiate between disturbances of sudden and more gradual onset by Cobbett (1), Lazarus-Barlow (2) and others and designated as shock and collapse respectively. Cannon, Cowell, Fraser and Hooper (3) divide these conditions of lowered blood pressure into primary and secondary shock. "Primary shock may come on so soon after injury as to be accounted for only as a result of nervous action" while secondary shock results after prolonged effects of inflicted injury, hemorrhage, exposure, etc. This research deals with secondary shock alone.

Shock has been defined as a disturbance in which the blood pressure has reached a level of about 50 mm. Hg. and has thus led more to work on the relation of blood pressure to the condition than on the relation of volume-flow of blood to the general nutrition obtaining.

---

[1] Reported in part before the American Physiological Society, Proceedings in this Journal, 1918, xlv, 545.

Volume-flow of blood appeared to be the more fundamental problem and as the results will show mean blood pressure gives very little evidence of the gravity of the disturbance. A primary rise in pressure occurring both in hemorrhage and tissue-abuse may be accompanied by a large decrease in volume-flow of blood, constituting the greater part of the total decrease reached by a subsequent fall to zero pressure.

Shock in this research is looked upon much as outlined in a previous report (4). It is considered in a very general way as a combined circulatory and nutritional disturbance resulting from a number of forms of tissue-abuse which if left to run their course may lead to death. Even though these forms of tissue-abuse, the initiating causes, may be removed the fatal issue is not necessarily avoided. Some changes apparently have taken place as a result of the tissue-abuse which sustains the condition. The factors in shock are therefore divided into *initiating factors* and *sustaining factors*.

The definition given by Lusk (5) explains what is meant by nutritional disturbance. "Nutrition may be defined as the sum of the processes concerned in the growth, maintenance and repair of the living body as a whole or of its constituent organs." The definition is accepted in this paper in its broadest sense.

Concerning the initiating and sustaining factors in shock, we know that experimentally a condition apparently comparable to shock as recognized clinically can be produced by numerous procedures. Shock is produced by mechanically inflicted injury, such as manipulation of the intestines or the crushing of muscle, by the effects of cold produced by immersion of the animal in iced water or the introduction of ice into the abdominal cavity, by burns or scalds, by over-anesthesia, by dehydration elicited by the introduction of hypertonic solutions into the abdominal cavity, by interference of the blood supply to the tissues accomplished by clamping of the inferior vena cava, clamping of the aorta and ligation of the veins from the extremities, by limitation of the oxygen intake, etc. For that reason forms of tissue-abuse which may be effective in producing shock are designated as initiating factors.

These initiating factors ultimately lead to changes in the tissues which have their influence on the "sum of the processes concerned in maintenance and repair" of living tissue. There is cellular damage, increased permeability of cells, transudation, eventual decrease in blood volume, slowing of the blood stream, etc. These represent sustaining factors some of which are not readily removed.

Since shock as recognized clinically is associated with mechanically inflicted injury, surgical or otherwise, it might be well to designate this injury as the *primary initiating factor* and other initiating factors which aggravate the condition, whether they were in operation before or after the inflicted injury, as *accessory initiating factors.* To the list should be added any form of tissue-abuse even though it alone may not, in the ordinary sense of the term, produce shock, e.g., thirst, fatigue, hunger, etc.

When the condition of shock is recognized at the front, the circulatory disturbances have reached a serious stage. Hence an important phase of the problem of shock is a matter of obtaining a basis for combating these circulatory disturbances.

There appears, however, to be another phase of equal if not greater importance, and that is the means of preventing these disturbances, a means of detecting the seriousness (6), (7), (8), of the initiating causes before the outstanding symptom of lowered blood pressure has developed, before serious structural changes have occurred and before irreparable damage has been done.

The emphasis laid on blood pressure previously expressed itself in the employment of pressor substances such as adrenin and pituitrin for the elevation of pressure at the expense of volume-flow. Though these forms of treatment have been largely discarded it appears that too much weight is still given to the significance of mean blood pressure relative to the conditions obtaining. Here lies a danger of permitting the development of shock which might otherwise be avoided. Although the animal itself employs pressor methods to combat a lowering of pressure, results have definitely indicated that a part of the problem of treatment and prevention of shock lies in the combating of these pressor reactions of the animal.

Though a blood pressure of normal magnitude is essential under normal conditions to maintain proper nutrition, and though blood pressure per se may be a factor of importance in the physiology of secretion, lymph, etc., yet we know that conditions of lowered blood pressure produced by transection of the cervical cord are survived. The survival is probably due to the fact that the lowered blood pressure is a result of vasodilatation, permitting an adequate volume-flow of blood.

Though the study of shock is at present receiving more attention, the common observation that "hemorrhage is the most potent shock producing factor" calls for a close comparison of the two conditions.

Hemorrhage and shock have many points of similarity. It appeared to me that the study of hemorrhage was important not only in itself, but especially in its relation to shock.

To be sure, certain outstanding features of the two conditions should call for different forms of treatment, but if these conditions are found closely comparable, if factors controlling the volume-flow of blood operate in a similar fashion, there might be a common basis for treatment and we would have at our disposal an easy and perfectly controllable method of abusing tissue and producing shocklike conditions amenable to the study of blood substitutes and requiring a minimum amount of aseptic surgery.

In these experiments shock was produced by exposure and manipulation of the abdominal viscera.

The data deal with:

a. Basal and superbasal-flow of blood in relation to metabolism.

b. The dependence of normal metabolism on basal blood-flow as measured by decreased alkaline reserve, etc.

c. A comparison of the basal-flow accompanying decreased blood pressure produced by hemorrhage and by tissue-abuse.

d. A comparison of the superbasal-flow of blood obtaining with lowered pressure from hemorrhage and tissue-abuse.

e. The relation of alkaline reserve to volume-flow of blood in hemorrhage, tissue-abuse and acid injection.

f. The respiratory function during hemorrhage, tissue-abuse and acid injection.

g. Comparative effects of gum acacia injection on the basal-flow of blood after hemorrhage and tissue-abuse.

The relative importance of the sustaining factor of slowed circulation depends on the degree to which tissues withstand a reduction in volume-flow of blood without injury. The detrimental effects of a marked decrease in flow accompanying a fall in pressure are easily demonstrable, but the effects of a smaller decrease in volume-flow unaccompanied by a change in pressure are not so apparent. Thus a demonstration of the dependence of normal metabolism on a normal flow of blood is desirable.

Data bearing on this point were obtained from experiments in this and the preceding paper (9) on *a*, the dependence of volume-flow of blood on metabolism; and on *b*, the dependence of metabolism on the volume-flow of blood.

a. Basal and superbasal-flow of blood as affected by metabolism. If we accept the view that liberation of metabolites increases the flow of blood on tissue activation, the experiments of the preceding paper show that the mechanism of volume-flow of blood is finely adjusted to metabolism and suggest that a normally increased function requires an increased volume-flow. It will be recalled that a stimulus too weak to elicit a visible secretion but strong enough to produce an electrical deflection, is accompanied by a considerable increase in volume-flow of blood. The linear relation of superbasal-flow to superbasal-metabolism whether dependent on the action of dilator fibers or dilator metabolites suggests a purposive mechanism of volume-flow control. These observations along with others, e.g., the slower recovery of a gland from activation when a low blood pressure prevails, the after-flow of blood following the release of arterial obstruction, etc., all point to a relatively narrow margin of safety of decrease in volume-flow.

b. Dependence of the tissues on basal-flow of blood as measured by decreased alkaline reserve. The experiments cited on the relation of volume-flow of blood to metabolism suggest a close dependence of the tissues on volume-flow, but more direct experiments were desirable. Knowing that a decreased oxygen supply to the tissues leads to the formation of acids other than carbonic acid and that these acids reduce the alkaline reserve, we might follow in a quantitative way the extent to which decreased volume-flow of blood affects oxidations.

Samples of blood were taken at regular intervals—first, during a normal control period with a constant volume-flow; second, during a period of reduced flow produced by hemorrhage; third, during another period of further reduced flow from a second hemorrhage. (See fig. 1.) The alkaline reserve of the blood was determined with the Van Slyke method (10) at forty-five minute intervals and plotted on the ordinates against time on the abscissas.

During the period of normal volume-flow the alkaline reserve remains approximately constant. During the next two periods, initiated by hemorrhage, the gradient takes two sharp dips, indicating that the disturbance in oxidations varies directly with the decrease in volume-flow of blood.

Objection to this conclusion might be raised on the ground that the decrease in alkaline reserve is attributable to a dilution of the blood by tissue fluids poor in alkali (11). To determine the importance of this factor in the dog the percentage of hemoglobin was likewise plotted.

The curve shows the effect of dilution, assuming the tissue fluids entering the blood to be free from alkaline reserve. The relative dipping of the two gradients therefore, indicates that the dipping of the CO_2 gradient is to be attributed mainly to decreased oxidations. This becomes more apparent through the observation that a decreased alkaline reserve of the blood is partially compensated by attraction of tissue alkaline reserve into the blood. (See page 482.) The degree to which this occurs may possibly account for the variability noted in the configuration of the curves, for all the curves do not show the sharp bending of the gradient or the perfect alignment of observations noted in figure 1. The attraction of alkaline reserve into the blood produces a bending of the curve upward and partly conceals the effects of decreased volume-flow of blood.

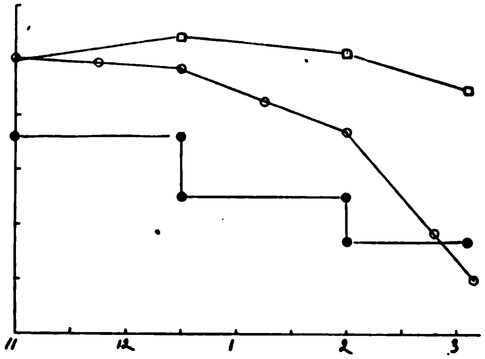


Fig. 1. Curves showing the relation of oxidative processes to volume-flow of blood. Hemoglobin percentage, carbon dioxide capacity and blood flow plotted on the ordinates against time on the abscissas.

Upper curve, hemoglobin percentage; middle curve, carbon dioxide capacity; lower curve, volume-flow of blood.

The detrimental effects of decreased flow are also shown in figures 3 and 4 where the variability of the factor of safety becomes evident. Take, for example, experiments 87 and 103 in which the volume-flows are markedly reduced, but apparently well borne as indicated by the horizontal CO_2 gradient. The interesting feature is that when the break in the CO_2 and respiratory gradients occurs it is abrupt. In experiments 85, 99 and 102, the deflection of the CO_2 and respiratory gradients follows promptly the decrease in volume-flow indicating an exceedingly small factor of safety. The experiments apparently represent conditions favorable for the development of shock.

c. A comparison of basal-flow of blood accompanying decreased blood pressure produced by hemorrhage and by tissue-abuse. If oxidations are as closely dependent on volume-flow as these experiments indicate, a reliable index to the flow of blood in shocklike conditions is desirable. The criterion most commonly used for gauging the circulatory disturbance in experimental shock is mean blood pressure. As was shown in the preceding paper, mean blood pressure in itself gives very little information on the volume-flow of blood obtaining. Unless blood pressure per se is of considerable importance in functions other than volume-flow of blood, it is a very misleading criterion of the condition of the animal.

The danger of gauging volume-flow of blood by mean blood pressure alone is due mainly to the double relation of pressure to the caliber of vessels—a decrease in caliber may be the cause of a rise in pressure and a fall in pressure may be the cause of a decrease in caliber. At least we know that in the submaxillary gland with the vago-sympathetic cut, vasoconstriction accompanies the primary fall in blood pressure. Perfusion of dead organs likewise shows a passive constriction during the early decrease in head of pressure.

Note that in figure 4, experiment 98 and figure 7, experiment 40, a large decrease in volume-flow of blood occurs during a perfectly stationary blood pressure. In experiment 87, figure 4 and experiment 85, figure 3, hemorrhage initiates a rise of blood pressure probably due to an over-constriction.

In figure 4, experiment 87, hemorrhage produced a rise in mean blood pressure from 112 to 116 mm. Hg. and decreased the volume-flow of blood 61.6 per cent of the original flow. The further fall from the augmented pressure of 116 mm. to 50 mm. Hg. produced a relatively slow decrease in volume-flow, only 17.8 per cent of the initial flow.

In figure 3, experiment 85, the rise in pressure is greater (11 mm., from 116 to 127 mm. Hg.) and the decrease in volume-flow is likewise greater—65 per cent. A point of special interest in this experiment is the sudden checking of the decrease in volume-flow during the rise in pressure which is changed to an increased flow during a fall in pressure.

The practical significance of mean blood pressure[2] as a gauge to the general condition is apparent: a constantly maintained pressure may be accompanied by a large decrease in volume-flow; two identical pressures may occur at different stages, one representing normal flow and the other a marked circulatory disturbance; a rise in pressure may be accompanied by a decrease in volume-flow of blood from two to three times as great as that accompanying the subsequent fall to zero pressure. The important point common to all observations is that the greatest decrease in volume-flow of blood occurs during a relatively small deviation from the initial pressure and that subsequent to this sudden decrease in volume-flow a further decrease in pressure of 80 mm. Hg. or more may be accompanied by only a relatively small decrease, no decrease or even an increase in volume-flow of blood.

---

[2] The relation of pulse pressure to the circulatory disturbance was not studied.

The circulatory disturbances, so far as the general volume-flow of blood is concerned, may be potentially as bad with a high as with a low blood pressure. The main difference in the two conditions is that the high pressure may represent a disturbance of a shorter duration and for that reason be more amenable to treatment. But in so far as the brain and heart consume more oxygen than the other tissues, and in so far as vasoconstriction of central origin may not reduce the flow of blood through these structures, as through other structures, the maintenance of a high blood pressure may be of some significance.

Usually the large and sudden decrease in volume-flow is reached before the pressure has fallen to a level of 90 mm. Hg. In one experiment on record, hemorrhage raised the initial pressure from 130 mm. to 140 mm. Hg. and when the initial pressure was subsequently reached the volume-flow was decreased 78 per cent. Therefore, pressures from about 140 or even higher to 90 mm. Hg. may be of little value regarding the volume-flow of blood and the general condition obtaining unless a previous record of pressure is at hand.

Figures 3, 4 and 7 show the similarity of the curves resulting from hemorrhage and from tissue-abuse. It appears, therefore, that fundamentally the same factors controlling volume-flow of blood are operating in the two conditions. These factors have been considered in some detail in the preceding paper on volume-flow of blood (12).

The well-known active constriction in hemorrhage may be looked upon as a reaction to decreased blood volume. Pilcher and Sollmann (13) refer the constriction to decreased oxidations in the vasomotor center resulting from the decreased volume. I am inclined to feel that the reaction may be of the nature of a volume-reflex similar to the lowering of blood pressure through the depressor nerve. This is suggested by the fact that although large and even rapid hemorrhages may occur without a lowering of the pressure yet constriction results. But this is not the question in which we are interested at present.

Protocols of several experiments are given to show the effects of hemorrhage. What concerns us in these protocols is not the maximum amount of bleeding a normal individual may withstand and still recover but rather the minimum amount producing a decided decrease in volume-flow of blood. These data are needed to ascertain whether we may have serious constriction of a similar source from transudation in tissue-abuse and to determine the relative importance of a decrease in blood volume whether it arises from hemorrhage or transudation both in initiating and sustaining shock.

Experiment 85. Repeated hemorrhages.

Weight of dog, 12,000 grams.

Blood volume, 1,080 cc.

No. 5. Total amount bled, 100 cc., about 9.3 per cent of blood volume. Total reduction of volume-flow, 49 per cent.

No. 9. Total amount bled, 195 cc., about 18.0 per cent of blood volume. Total reduction of volume-flow, 65 per cent.

Experiment 87. Repeated hemorrhages.

Weight of dog, 14,940 grams.

Blood volume, 1,340 cc.

No. 3. Total amount bled, 115 cc., about 8.6 per cent of blood volume. Reduction of volume-flow, 61.6 per cent.

Experiment 103. Repeated hemorrhages.

Weight of dog, 18,300 grams.

Blood volume, 1,675 cc.

No. 5. Total amount bled, 175 cc., about 9.6 per cent of blood volume. Total reduction of volume-flow, 42.2 per cent.

No. 7. Total amount bled, 275 cc., about 15 per cent of blood volume. Total reduction of volume-flow, 44.8 per cent.

Experiment 46. Repeated hemorrhages.

Weight of dog, 15,800 grams.

Blood volume, 1,420 cc.

Total amount bled, 150 cc., about 10.5 per cent of blood volume. Total amount bled, 360 cc., about 25 per cent of blood volume.

Total reduction of volume-flow, 69.5 per cent.

Experiment 49. Continuous hemorrhage through the vessel of the gland.

Weight of dog, 13,250 grams.

Blood volume, 1,196 cc.

Total amount bled, 539 cc., about 45 per cent of blood volume.

Reduction of volume-flow, 70 per cent.

Experiment 48. Continuous hemorrhage through the vessels of the gland.

Weight of dog, 12,500 grams.

Blood volume, 1,135 cc

Total amount bled, 325 cc , about 28 per cent of blood volume.

Total reduction of volume-flow, 42 per cent.

Total amount bled, 474 cc , about 42 per cent of blood volume.

Total reduction of volume-flow, 66 per cent.

Observe the varying effects of hemorrhage. The smaller decrease in volume-flow elicited by a continuous hemorrhage agrees with the observation that animals withstand slow hemorrhage better than more rapid hemorrhage. But another explanation is possible. The relative and absolute decrease in volume-flow resulting from reduced blood volume depends upon the constrictor tonus at the beginning of the experiment. If for any reason that is high, (from previous concentration, for example) the effects of lost volume are reduced. For a specific case refer to experiment 100, page 478. Had the initial determination of volume-flow been postponed but a few minutes the major effects of decreased volume would have been missed. It is probable that in most cases the volume-flow decreased more during the course of the experiment than is represented in the protocols and graphs. But even disregarding such possibilities the percentage decrease of volume-flow of blood far exceeds the percentage decrease in blood volume in every experiment. The maintenance of a normal blood volume is, therefore, an extremely important function.

The question arises—does the same mechanism reduce volume-flow in tissue-abuse?

From the observations of Sherrington and Copeman (14), Cobbett (15), Lazarus-Barlow (16), Vale (17), Gasser, Meek and Erlanger (18) and others we know that concentration of red cells in the blood is a striking feature of tissue-abuse. Occasionally, even in the early periods of an experiment I have noted a diminution in blood volume from this source amounting to more than 20 per cent. Cobbett records a reduction of 30 per cent. Employing the gum acacia method of Meek and Gasser (19) for determining blood volume, Gasser, Meek and Erlanger find an average reduction of blood volume amounting to 17.6 per cent. The decrease of volume-flow in tissue-abuse is, therefore, readily accounted for in most instances.

Though the vasoconstrictor center is very sensitive to small changes in blood volume, slight reduction in the early stages of tissue-abuse might appear insufficient to elicit the decreased volume-flow. The experiments of Pilcher and Sollmann showing that a shunting of blood to a local area by local dilatation produces a general vasoconstriction meets this difficulty for the viscera, after manipulation, give every appearance of acute inflammation with an increased blood flow. The effects on constriction of decreased circulating blood from stasis should also be mentioned (Welch, Mann, and Erlanger and Gasser).

Only rarely can the effect of reduced blood volume from tissue-abuse be studied as clearly as in hemorrhage. Therefore experiment 100 is described in some detail, an experiment in which for some unknown reason rapid and marked concentration of the blood occurred with the animal almost intact even before the abdomen was opened. The animal experimented on was a male bull dog apparently in perfect health, previously injected with morphine sulphate which had taken marked effect. The dissection about the submaxillary gland was possible without the use of ether.

The volume-flow of blood was 100 drops per minute and the volume percentage of red cells was 60. To eliminate effects other than manipulation of the viscera, which was to follow, another determination of the blood flow was made one half-hour later and found to be 30 drops per minute Not knowing how to account for such a striking reduction in volume-flow, the blood pressure having remained constant at 130 mm. Hg. and the dissection of the gland having been a very easy one, the percentage o' red cells was again determined and found to be 79. The diminution in blood volume which such concentration entails accounts for the serious constriction. That such disturbances occur without any outward signs whatever suggests an explanation of some of the more mysterious cases of shock. The experiment shows again the danger of relying too much upon blood pressure measurements.

That general constriction rather than exhaustion of the vasomotor center exists in the early stages of shock is generally accepted, (20), (21), (22), (23), (24), (25), (26), but experiments such as no. 100 point definitely to the source of the reaction—a decreased blood volume similar to the source of constriction in hemorrhage.

Epstein (27) calls attention to concentration of red cells from administration of ether. Whether the small amount used in experiment 100 could account for the transudation of plasma is questionable, however.

It might be mentioned in connection with decreased blood volume that manipulation of the intestines in many animals produces a prompt and visible thickening of the intestinal wall and cessation of manipulation is frequently followed by considerable recovery of the normal thickness. These events may be frequently repeated; and they presumably are accompanied by fluctuations in blood volume as is indicated by synchronous oscillations in mean blood pressure.

I also have observed that manipulation of limited segments of the intestines though initially leading to a definite fall in pressure soon becomes less effective. This may be due to the fact that maximum transudation in the manipulated segment has occurred for manipulation of a new segment now leads to further transudation and fall in pressure. Other explanations of course are possible.

All the experiments indicate the similarity of the vascular reactions in hemorrhage and tissue-abuse. This is borne out by the following observations. Pilcher and Sollmann noted that animals with a low initial pressure withstand hemorrhage far better than those with a high pressure, a fact also noted in the present research. The manner in which some animals withstand large and even rapid hemorrhages without a lowering of the pressure during bleeding is remarkable. The counterpart of this is the observation of Erlanger and Gasser that animals with a high initial pressure respond less well to treatment after clamping of the cava than do animals with a lower initial pressure.

d. A comparison of the superbasal-flow of blood with lowered blood pressure from hemorrhage and tissue-abuse. In the regulation of volume-flow of blood to the tissue needs we are not only interested in the manner in which the flow to a resting tissue varies with changing head of pressure, but also in the superbasal-flow for activated tissue. Undoubtedly in the marked nutritional disturbances occurring in conditions of lowered blood pressure, tissues suffer not only from decreased basal-flow but likewise decreased superbasal-flow. That the liver, kidney and probably the heart are called upon for increased activity seems possible.

By stimulating the chorda tympani so as to elicit equal amounts of saliva with varying heads of pressure and measuring the extra flow of blood per drop of saliva we determined the conditions under which tissues work in hemorrhage. (See page 451, paper III) (28). It was found that superbasal-flow divided by superbasal-metabolism decreases out of all proportion to the decrease in pressure.

The effects of tissue-abuse are equally as great as hemorrhage (see fig. 2 and table 1). In figure 2 the basal-flow of blood is plotted against mean blood pressure in the usual way, and observations shown in the table are designated on the curve.

The initial changes in blood pressure have the most detrimental effects on superbasal-flow as well as on basal-flow. Though a 100 mm. Hg. might be considered a normal pressure for some dogs, yet when that pressure is reached in this experiment the superbasal-flow of blood is more than halved. Since the stretching head of pressure seems adequate to take advantage of any inhibition of tonus and produce an adequate superbasal-flow of blood we are reminded of the experiments of the preceding paper of combined and separate stimulation of the chorda tympani and vago-sympathetic (see fig. 4, paper III).

The amount of secretion obtained by combined stimulation is greater than that obtained by chorda stimulation alone, and yet is accompanied by a smaller volume-flow of blood. This smaller volume-flow was attributed to the failure of the chorda effects to inhibit the increased tonus resulting from vago-sympathetic stimulation.

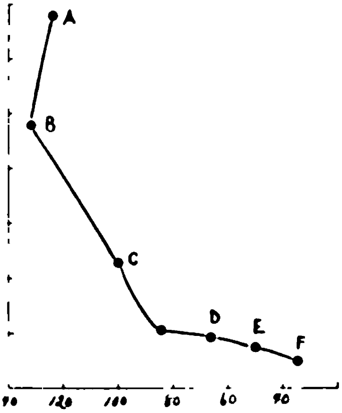


Fig. 2. Basal-flow of blood, from an experiment on the effects of tissue-abuse plotted on the ordinates against mean blood pressure on the abscissas.

TABLE 1

OBSERVATION	BLOOD PRESSURE IN MM. HG.	BASAL FLOW OF BLOOD IN DROPS PER MINUTE	SECRETORY BLOOD FLOW DIVIDED BY SECRETION
A	124	68.0	13.9
B	132	48.0	12.0
C	100	23.0	5.9
D	66	9.5	3.3
E	50	7.7	1.8
F	35	5.0	0.0

During the earlier stages of hemorrhage and tissue-abuse we have comparable conditions; an increased constrictor tonus which must be inhibited before a superbasal-flow of blood can take place. That this is a factor of some significance is indicated in figure 2 and table 1, for despite the increased stretching head of pressure at *B*, the increased constrictor tonus which is the cause of the rise in pressure is not as thoroughly inhibited and the superbasal-flow of blood is decreased.

The danger that such a mechanism may act detrimentally on some important activated tissue is apparent.

e. The relation of alkaline reserve to volume-flow of blood in hemorrhage, tissue-abuse and acid-injection. The relation of volume-flow of blood to alkaline reserve is a perplexing one.

It will be recalled (29) that with innervation intact acid injection may decrease the basal-flow of blood and with the vago-sympathetic severed elicit increased flow; that the superbasal-flow of blood appears at times to be increased by a decreased alkaline reserve, and that a possibility of a balanced action, peripheral and central exists.

From a practical point of view, I have nothing definite to add but some observations may be of significance.

Animals withstand large injections of acid which may lead to an increased rather than decreased pressure. An increased pressure of 140 mm. Hg. at a Van Slyke reading of 6 giving way suddenly to zero pressure on further injections indicates that the heart may for a time at least execute extra work with an exceedingly low alkaline reserve.

But that the organism strives to prevent a development of a low alkaline reserve long before that is reached is shown in table 2; n/2 hydrochloric acid was injected intravenously at intervals of approximately 30 minutes and the CO_2 content followed. (This experiment was performed for another purpose, therefore the intervals and amount of acid are not the same. The results, however, are so striking that the conclusions are not affected.) The CO_2 content was determined prior to each injection. The reduction of alkaline reserve with each injection divided by the amount of acid injected gives the effectiveness of the acid to neutralize the reserve at different stages of the experiment. The effectiveness rapidly decreases and at the end the tissue reserve enters the blood so rapidly that the acid injection in the interval allowed is without effect.

The wide variations of alkaline reserve noted by Henderson and Haggard (30), and by Scott (31) and their relation to morphinization were observed by me. In addition it was found that the lethal injection of acid per kilo of body weight was less in animals with a high alkaline reserve than in animals with a low alkaline reserve.

The passage of alkaline reserve into the blood may represent one of the normal mechanisms of combatting increased hydrogen ion concentration yet as Bayliss (32) pointed out a low alkaline reserve may not be without its advantages when the effects on volume-flow of blood, gaseous exchange in the lungs and in the tissues in general are considered. Though the results of the preceding paper suggested similar ideas, a maintenance of normal alkaline reserve is probably preferable. Erlanger and Gasser find that injection of carbonate in concentrated form is distinctly harmful but is not harmful when injected in weaker concentrations. In the matter of treatment Bayliss and Erlanger and Gasser feel that with improved circulation the alkaline reserve will take care of itself. The rapid passage of alkaline reserve into the blood as shown in table 2 illustrates how readily a lowered alkaline reserve is overcome. The work of Howell, Seelig, Mann and others, however, indicates the beneficial effects of injection of carbonate.

TABLE 2

$$\frac{\text{Van Slyke Readings}}{\text{Acid injected}} = \text{Effectiveness of acid to reduce the alkaline reserve}$$

$$\frac{51.8 - 42.4}{26} = 0.361$$

$$\frac{42.4 - 28.0}{50} = 0.288$$

$$\frac{28.0 - 20.0}{28} = 0.285$$

$$\frac{20.0 - 14.0}{30} = 0.200$$

$$\frac{14.0 - 9.7}{32} = 0.134$$

$$\frac{9.7 - 9.7}{19} = 0.000$$

f. The respiratory function during hemorrhage, tissue-abuse and acid injection. The similar and normal constrictor reaction of the vasomotor center in hemorrhage and tissue-abuse and the normal reaction of the cardio-inhibitory center in shock (33), suggested also the need of a closer comparison of the respiratory function in the two conditions than has hitherto been made. In such a study we are interested in the reaction of the respiratory center to the decreasing alkaline reserve prevailing in order to determine more nearly the relative importance of a general involvement of the medullary centers and the relation of the reaction to general oxidative processes.

In studying respiration we must distinguish between cause and effect. Van Slyke (34), Henderson and Haggard, Scott and others point to the readiness with which the alkaline reserve of the blood may shift. I have found the effects of morphinization pointed out by Henderson and Haggard and Scott to be striking and also that painful stimulation from tissue-abuse is likely to lead to over-ventilation with a reduction of the alkaline reserve of the blood.

It is therefore realized that a study of respiration is best carried out on a decerebrate animal. But the curves will show that under certain circumstances, with both acid injection and hemorrhage, a uniformity of results is obtainable. In tissue-abuse where painful stimuli enter into play such uniformity is not to be expected and therefore if only occasional experiments are comparable to those of hemorrhage we might be justified in concluding that the response to changing alkaline reserve would be similar were painful stimuli always excluded.

The general effect of decreased alkaline reserve in hemorrhage and tissue-abuse in its relation to respiration is shown in figures 3 and 4. The experiments on hemorrhage are designated by an *H* and on tissue-abuse by *T. A.* and are shown in pairs for purposes of comparison.

Respiration and carbon dioxide content of the arterial blood are plotted on the ordinates against mean blood pressure on the abscissas. A definite similarity in the increased response of the center to decreasing alkaline reserve appears, but a better comparison is obtained by plotting the respiration against carbon dioxide capacity as shown in figure 5.

To control the results obtained in hemorrhage and tissue-abuse the effects of lowered alkaline reserve by acid injection (N/2 HCl) were first studied. Plotting the results in the manner described we find the reaction of the respiratory center to be represented by a straight line. (See fig. 5). Apparently this may be looked upon as the normal response.

Reduction of the alkaline reserve by a decreased volume-flow produced by hemorrhage elicits the same reaction. It would seem that the decreased volume flow per se accompanying the decreased alkaline reserve is without effect upon the respiratory center. But as pointed out before, the curve of flow through the submaxillary gland may not represent the flow obtaining under similar conditions through the brain.

The rapid rise of the curves of respiration in experiments 98 and 91 on tissue abuse represent exceptions to the previous reactions noted and suggest a reflex excitation of the respiratory center by painful stimuli. The disturbance in oxidations could hardly be marked

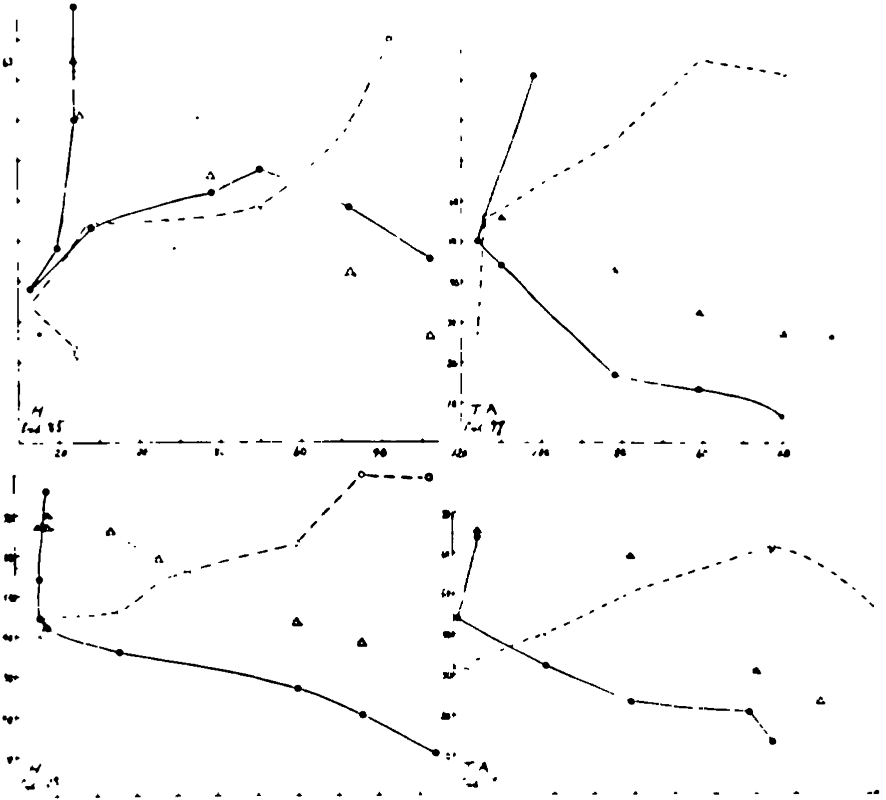


Fig. 3

Figs. 3 and 4. Comparison of conditions in experiments on hemorrhage, *H*, and tissue abuse, *T A*. Basal flow, ● respiration, O, carbon dioxide content of arterial blood, △ and hemoglobin percentage plotted on the ordinates against mean blood pressure on the abscissas □. The figures on the ordinates represent carbon dioxide content; on the abscissas mean blood pressure in mm. Hg.

enough to account for the sudden diminution in alkaline reserve. See also experiment 98 as plotted in figure 4.

In experiments, however, where pain stimuli for some reason are less effective, the response of the respiratory center appears to be normal (see exps. 101 and 90 in fig. 5). The earlier failure of the center noted in certain cases of shock may represent a reaction to decreased body temperature from visceral exposure, a factor not carefully controlled.

If this is true cold is important from many points of view, not only in reducing blood-flow and oxidation, (35), (36), but depressing the respiratory center as well and from these experiments we infer that where the factor of cold does not enter the danger of hemorrhage and tissue-abuse is not attributable to a failure of the respiratory center. It may be, however, that the more shallow and rapid respirations in tissue-abuse are not as effective in securing gaseous exchange as the slower and deeper respirations in hemorrhage. No effort was made to determine the reason for the difference in the type of respiration. Pos-

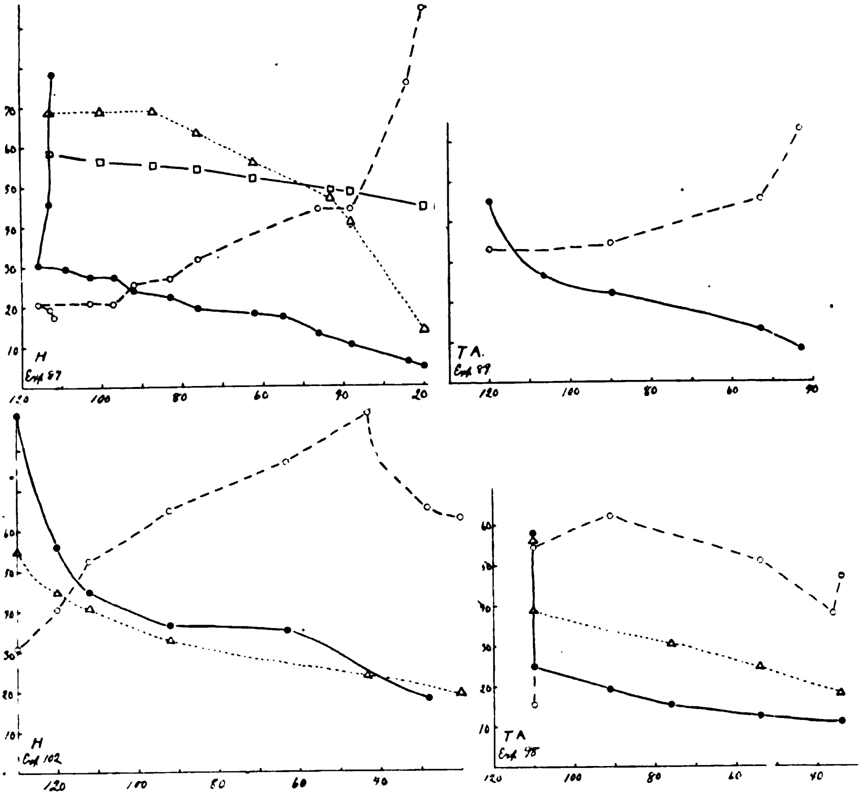


Fig. 4

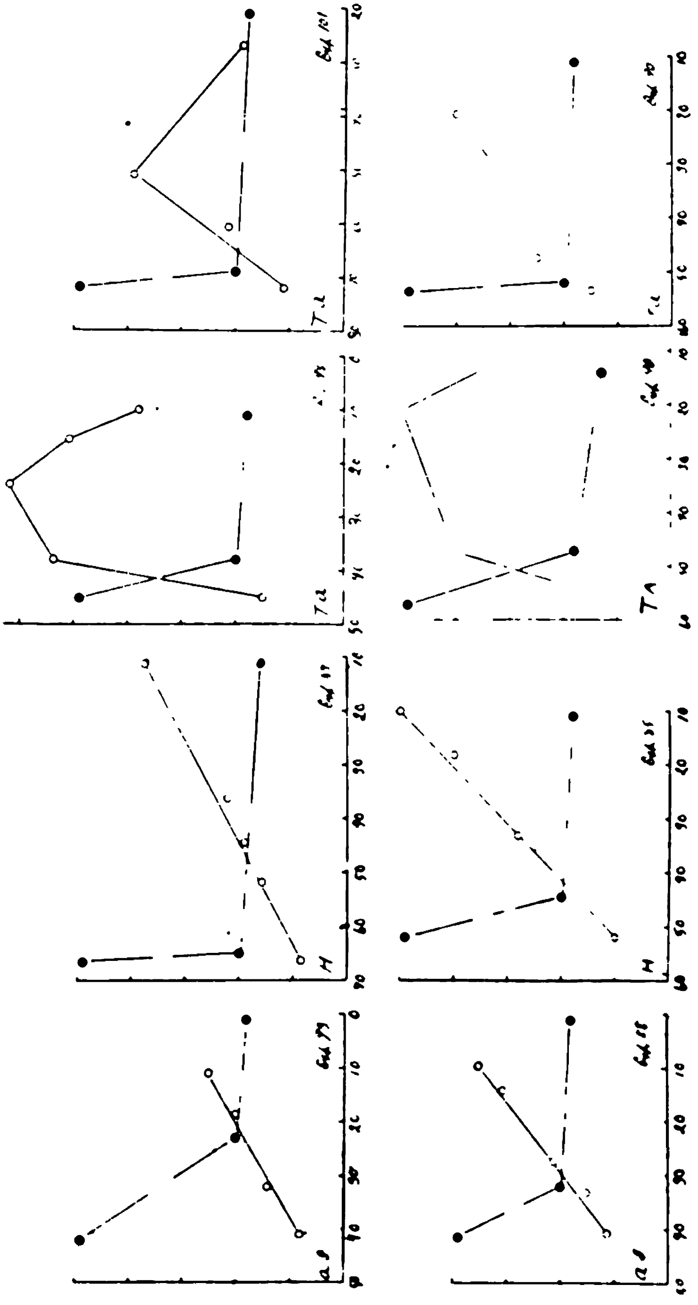


Fig. 5. Curves showing the response of the respiratory center to decreasing alkaline reserve in experiments on effects of acid injection, *A. I.*, hemorrhage *H* and tissue-abuse, *T. A.* Respiratory exchange is plotted on the ordinates against carbon dioxide capacity of arterial blood on the abscissas. Basal-flow of blood is roughly plotted on the ordinates.

sibly the opening of the abdomen interferes with the action of the abdominal respiratory muscles.

g. Comparative effects of gum acacia injection on the basal-flow of blood after hemorrhage and tissue-abuse. The experiments to be described were performed for a number of purposes:

1. To check the effects of inflammation on volume-flow of blood in the experiments on hemorrhage.
2. To determine the importance of the viscosity factor in volume-flow of blood in hemorrhage and in tissue-abuse.
3. To determine and compare in hemorrhage and tissue-abuse the manner in which volume-flow of blood responds to increased blood volume.
4. To arrive at some basis of treatment by taking into consideration the factor of volume-flow times the concentration of the blood.

g. 1. The similarity of the three curves of volume-flow in figure 6 obtained by bleeding, by acacia injection and by rebleeding indicate that the factors of cold and inflammation so far as they are concerned in the hemorrhage experiments have little influence on the volume-flow of blood. They show that severe hemorrhage if compensated in time is without serious effect upon the constrictor tonus and on the normal elastic properties of the vessels for the response is similar in three alterations of pressure. (See also curves obtained during bleeding and injection in fig. 7, exp. 49.)

g. 2. Changing the mean blood pressure by hemorrhage, by tissue-abuse and by acacia injection we get three combinations of varying pressures and concentrations of corpuscles. Assuming the corpuscle to be the main viscosity factor the curves of flow show that changes in viscosity occurring in these experiments have relatively little effect upon the configuration of the curve. The effects appear more upon its position.

g. 3. For example in experiment 49, figure 7, the injection curve differs from the hemorrhage curve mainly in position; this change is largely accounted for by dilution of the blood and by deterioration of the vasomotor center. The curve, however, represents a good response on the part of the vascular system to injections. The relatively insignificant effects of rise in pressure up to 85 mm. Hg. on the volume-flow and the enormous effects of a further increased pressure point to the practical importance of adequate injections in the treatment of lowered blood pressure. The shifting of the injection curve to the right indicates, however, that we should be satisfied in attaining pressures slightly below the normal pressure of the individual in question.

A prolonged condition of lowered blood pressure disturbs the normal response to injection. Apparently the vasomotor center and the elastic properties of the vessels are affected. The contour of the injection curve approaches a straight line. (See injection and rebleeding curve of exp. 48 and the rebleeding curve of exp. 49, fig. 7). This variation in response appears to be a matter of some importance relative to treatment of conditions of lowered blood pressure and will be considered below.

The experiments on the comparison of the response of the vascular system after tissue-abuse with the response in hemorrhage are limited Figure 7 however shows that considerable similarity in the two conditions may be expected.

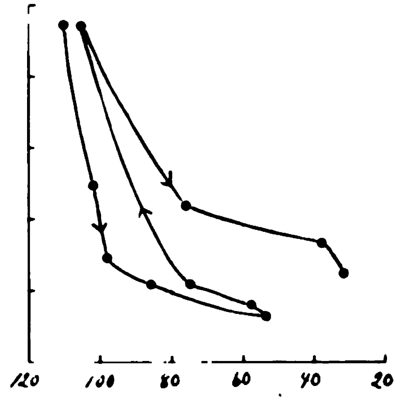


Fig. 6. Three curves of basal-flow of blood from hemorrhage, acacia injection and rebleeding.

Nevertheless, in certain instances (exp. 51, fig. 7) a shifting of the injection curve to the left occurred, an observation missing in the hemorrhage experiments, attributable I believe to the effects of a small fall in body temperature absent during hemorrhage.

g. 4. In the treatment of hemorrhage and shock transfusion both of blood and of inert solutions is practiced. So far as I am aware, apart from supplying fluid and fluid transport to the tissues, we have relatively little experimental basis for the treatment of conditions of lowered blood pressure by inert injection[3] and particularly hemorrhage and shock plus hemorrhage. The data we have is mainly of an empirical nature, namely, the beneficial effects following injections of saline solutions and of colloidal suspensions. It seemed, therefore, that if a basis for such treatment is found the procedures and details of administering injection might be improved. We would in addition have a basis for modifying the nature of the injection fluid to meet more nearly the needs of the tissues.

---

[3] By inert injection is meant the injection of a fluid so far as the tissues are concerned, physically and chemically inert, possessing the same colloidal and saline osmotic pressure and a similar viscosity as that of blood and having no chemically stimulating effect. A 7 per cent gum acacia suspension in 0.9 per cent NaCl may be looked upon as an approximation to such a solution.

Looking upon blood as a means of transport of nutrient material to, and of waste material and secretions from the cells, we may be justified in assuming that either a dilution of the blood or a reduction in the flow would interfere with both of these functions. It is probable that the availability of any nutrient to the tissues depends upon the combination of two factors—volume-flow times concentration of the nutrient in question particularly when deficiencies exist. This combination I have called the *nutrient-flow*.

The damage done by a decrease in the nutrient-flow probably depends upon the extent to which any essential nutrient (oxygen, salts, alkali, derivatives of fats, carbohydrates and proteins) is depleted as the blood flows through the tissues. An important question, therefore, is to determine the particular deficiencies leading to disturbed nutrition.

It appears that the oxygen supply to the tissues is of utmost importance. This is more recently borne out by the demonstration of the equal effectiveness of the injection of red cell suspensions and of whole blood (37) yet it might be mentioned that the cells in this case were suspended in glucose solution. The beneficial effect and advantages of injection of sugar solutions noted by Sansum and Woodyatt (38), Erlanger and Woodyatt (39), Erlanger and Gasser and others (40), (41), (42), (43) and (44), in shock and in other conditions indicate the desirability of increasing the sugar concentrations of the blood. The unsettled views on alkaline reserve call for further study of this factor. This phase of the subject will be considered in greater detail in the discussion.

It is apparent that the increased volume-flow produced by transfusion of whole blood within certain limits might be of value in direct proportion to the increased flow. The question arises—wherein lie the beneficial effects of inert injection? Can the nutrient flow of any constituent be increased without its addition to the circulating blood? If so, the flow of blood must increase out of proportion to the extent of dilution eliciting the increased flow. That this occurs follows from the previous observation that a decrease in blood volume produces a decrease in volume-flow out of all proportion to the decreased volume, and from the observation that the volume-flow curve with decreasing pressure may be duplicated with rising pressure from acacia injection.

The question, however, can be put to the direct test by measuring the volume-flow with decreasing and increasing pressure, multiplying by the percentage of red cells and obtaining the nutrient-flow. The data from four such experiments, two on hemorrhage and two on tissue-abuse, are shown in figure 7 and table 3. The data of the lettered observations are given in the table blood pressure, volume-flow, percentage of red corpuscles or nutrient-concentration, nutrient-flow,

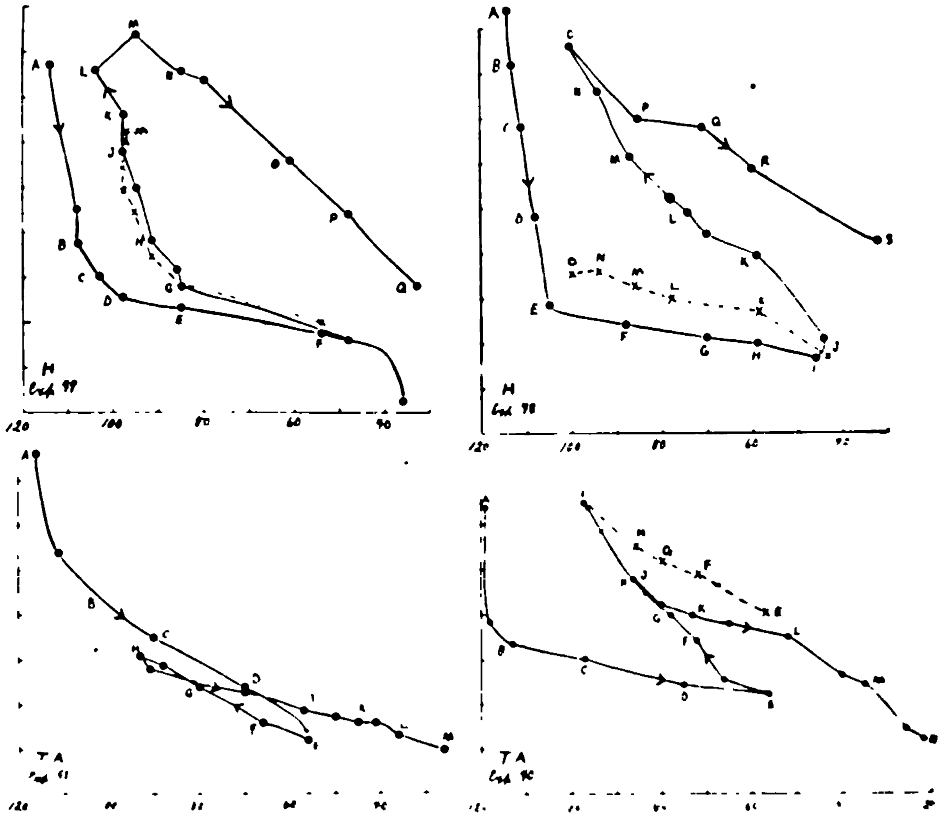


Fig. 7. Comparison of curves of basal-flow of blood in hemorrhage and tissue abuse showing the response of volume-flow to injection of acacia. With table 3 it illustrates the changes in nutrient-flow accompanying changes in volume-flow; the curve of nutrient-flow is represented by crosses. The curve nutrient-flow in experiment 51 follows the injection curve of flow.

TABLE 3

Hemorrhage

EXPERIMENT 48

Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow
A	114	94.0	55.6	5195	100.0	
B	113	82.0	54.6	4476	86.3	
C	111	68.0	52.9	3592	69.2	
D	108	48.2	51.8	2497	48.3	
E	105	28.7	50.5	1449	27.9	
F	88	24.2	49.3	1193	22.9	
G	70	21.3	49.0	1043	20.1	
H	59	20.0	48.8	976	18.8	
I	46	16.5	48.4	798	15.3	100.0
J	44	21.0	41.7	875	16.9	112.7
K	59	39.5	36.2	1430	27.6	184.1
L	74	49.0	32.0	1568	30.2	201.2
M	87	61.5	28.1	1727	33.3	222.0
N	94	76.0	24.9	1893	36.5	240.0
O	100	86.0	21.9	1884	35.5	236.7
P	85	70.0	21.4	1498	28.9	192.7
Q	71	68.0	20.9	1421	27.4	182.7
R	60	59.0	20.3	1197	23.0	153.5
S	32	43.0	16.2	688	13.2	86.7

EXPERIMENT 49

Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient-concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow
A	114	77.0	52.2	4020	100.0	
B	108	37.0	51.8	1916	47.6	
C	103	30.0	50.2	1506	37.5	
D	98	25.6	50.0	1412	35.1	
E	85	23.4	48.7	1139	28.3	
F	84	17.5	47.0	822	20.4	100.0
G	85	28.0	40.2	1124	27.9	142.5
H	91	38.0	37.3	1417	35.2	179.5
I	95	50.0	35.8	1791	44.5	227.0
J	98	58.0	34.4	1991	49.5	252.3
K	98	66.0	32.9	2171	54.0	275.3
L	104	76.0	32.0	2431	60.5	309.0
M	95	84.0	30.0	2520	62.7	320.0
N	85	76.0	28.5	2165	53.9	275.0
O	61	56.0	24.2	1355	33.7	172.0
P	48	54.0	23.4	1029	25.6	130.7
Q	33	28.0	21.7	604	15.0	76.5

Tissue-abuse

EXPERIMENT 40

Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow
A	119	64.0	60.0	3840	100.0	
B	118	38.5	67.0	2578	67.2	
C	97	30.5	70.0	2134	55.6	
D	75	24.5	73.0	1788	46.6	
E	56	22.0	72.0	1584	41.2	100.0
F	72	34.5	54.4	1875	48.8	118.5
G	78	40.0	49.8	1992	51.9	125.7
H	78	48.0	44.0	2112	55.0	133.5
I	97	65.0	37.9	2462	64.2	156.0
J	85	47.0	37.0	1740	45.3	110.0
K	73	40.0	36.4	1455	37.9	92.0
L	52	33.0	35.7	1178	30.7	74.5
M	35	24.0	34.1	816	21.2	51.4
N	22	12.0	33.1	396	10.3	25.0

EXPERIMENT 51

Observation	Blood pressure	Basal-flow	Percentage of red cells or nutrient-concentration	Nutrient-flow	Nutrient-flow relative to initial nutrient-flow	Nutrient-flow relative to lowest nutrient-flow
A	116	76.0	65.6	4985	100.0	
B	100	46.0	70.4	3235	65.0	
C	90	35.0	70.6	2471	49.6	
D	70	24.0	73.2	1757	35.2	
E	56	12.0	69.4	832	16.7	100.0
F	66	16.0	60.6	969	19.4	116.0
G	80	23.0	55.3	1265	25.4	152.0
H	92	31.0	48.6	1504	30.2	181.0
I	80	25.0	44.4	1110	22.3	133.5
J	57	18.7	43.4	811	16.3	97.6
K	45	16.0	42.4	675	13.5	80.8
L	36	13.0	41.3	537	10.8	64.6
M	26	9.8	42.8	419	8.4	50.2

nutrient-flow compared with the normal initial nutrient-flow valued at 100, and the nutrient-flow relative to the lowest nutrient-flow also valued at 100. The curves of nutrient-flow as well as of volume-flow are shown in figure 7.

In experiment 49 on hemorrhage the pressure was lowered to 36 mm. Hg. The nutrient-flow was decreased to less than 20 and was raised again by acacia injection to 62.7. The value of the highest nutrient flow at *M* compared with that at *F* is 309.

In experiment 48, also on hemorrhage, the nutrient-flow was decreased to 15.3 but subsequently raised only to 36.5.

The two experiments on tissue-abuse also represent what might be looked upon as a successful and less successful treatment. In one case the nutrient-flow was lowered to 41.2 and raised to 64.2, in the other lowered to 19.4 and raised to 30.2. The failure to improve the nutrient-flow more in these experiments is largely due to a failure to inject enough acacia. For example, in experiment 51 the percentage of corpuscles at the height of injection was 48.6. Further dilution undoubtedly would have been accompanied by an increased nutrient flow.

It is apparent from these tables that in both hemorrhage and in tissue-abuse the condition of lowered blood pressure may be markedly improved by the injection of an inert solution.

In attempting to explain the increased nutrient-flow accompanying increased volume-flow from inert injection the factor of pressure is practically eliminated by analysis of conditions represented in figure 7 experiment 49, where pressure remains constant but volume-flow rapidly increases. By assuming the function of the heart to remain constant the dependence of volume-flow is reduced to three main factors - viscosity, caliber of the vessels, and filling of the heart. All of these factors are undoubtedly operative in increasing the flow.

Concerning viscosity, Tigerstedt noted that relatively large injections of defibrinated blood affected little the ventricular output of the rabbit while similar injections of salt solution augmented the flow. The difference in results he attributed to the diminution of viscosity of the blood by saline injection. This effect of decreased viscosity is also noted in the shifting of the injection curve of volume-flow to the right in figure 7 of this paper. But if this factor is operative in increasing the nutrient-flow it must decrease the viscosity out of proportion to the dilution entailed. An analysis of the tables of Burton-Opitz would indicate this as a possibility if the results of viscosity as measured by flow of blood through a glass capillary tube can be applied to the peripheral flow of blood in situ. Though a change in viscosity may influence the nutrient as well as the volume-flow it does not appear to be a factor when gum acacia is injected, as is indicated by the relatively small effect of change in viscosity (as measured by the corpuscles) on the curves of volume flow. This is further borne out by the relatively small increase in nutrient and volume-flow elicited by acacia injection in experiment 48 when compared with experiment 49.

Some other factor comes into play. In later experiments controlling the effects of small intravenous injections of drugs on cardiac output, Tigerstedt noted that an increase of the normal blood volume augmented the cardiac output out of proportion to the injection. The curves of volume-flow of blood of the submaxillary gland show that in all stages of depleted blood-volume injection of inert solution may increase the volume-flow out of all proportion to the dilution produced. We know from the increased filling of the heart which occurs that dilution results both from the increased capacity of the heart and of the larger vessels, and from the increased volume-flow occurring with constant head of pressure that dilution must also result from the increased capacity of the peripheral vascular system. It is of interest, therefore, to know how the nutrient-flow increases despite these two dilutions.

The explanation of this phenomenon, I believe, is to be found in the application of Poiseuille's law for the flow of fluids through tubes, in conjunction with changes in the capacity of the vascular system accompanying increased blood flow. By experiments upon the flow of distilled water in capillary tubes 0.15 to 0.65 mm. in diameter Poiseuille reached the conclusions represented in the formula $A = \frac{kR^4}{L} H$. A, volume outflow per second, R radius and L, length of the tube, H, the pressure head, and k the transpiration coefficient.

Assuming the length of the peripheral vessels directly determining the volume-flow to remain constant the conclusion in which we are particularly interested is that with the same head of pressure the time spent in the outflow of a certain volume of fluid through equally long tubes is inversely proportional to the fourth power of the diameter of the tube. Assuming further that the capacity of the vascular system increases only through increased caliber of the peripheral vessels it must follow that in the increased volume-flow and the increased caliber of the vessels occurring in the sharp ascent of the curve of basal-flow during injection, that the volume-flow increases directly as the fourth power of the caliber of the vessels while the volume of the vessels or the dilution increases only as the second power of the caliber.

A careful application of this relation has not been made but it is obvious that in the relation of the fourth to the second power great possibilities for improvement of nutrient-flow by simple dilution exists. For working out these possibilities, however, we need information on the diluting effects of increased capacity of the larger vessels produced by injection. The relative significance in changes in the arteriole and capillary bed must also be considered.

It will be noted that the nutrient-flow increases during the early stages of injection while the peripheral vessels are contracting as well as in the later periods where dilatation occurs. The increased nutrient-flow may be explained by bearing in mind the effects of increasing head of pressure and the observation of Pilcher and Sollmann that at the lower pressures small volume changes are most effective in producing pressure changes.

Though the experiments described represent only a preliminary study on nutrient-flow they suggest some points of significance in treatment.

Note that the experiments on hemorrhage and tissue abuse in which nutrient-flow was most improved are those in which the injection curve of flow more nearly approximates the first curve. In these instances the decreased flow preceding injection has had relatively little effect upon the vasomotor center and on the elastic properties of the blood vessels. Note also that little effect on nutrient-flow is obtained until a relatively high pressure is reached when a sudden increase occurs; emphasizing the importance of not only attaining a high pressure but of increasing the blood volume still more to overcome the vasoconstriction.

If the decreased flow is prolonged and has resulted in greater changes in the vessels and in the vasomotor center as suggested in experiment 48, the relation of volume-flow to increased blood volume is much less favorable for successful treatment. The normal response of volume-flow to injection is missing, the amount of dilution approaches more nearly the increased volume-flow of blood and the result is a relatively smaller improvement in the nutrient-flow.

The more rapid increase in volume-flow with an elevation of pressure at the lower levels indicates a failure of the peripheral vessels to contract and may represent a general failure of the vascular system which requires for a given increase in volume-flow a greater dilution of the circulating blood, thereby counteracting the beneficial effects of dilatation previously outlined. We are reminded here of the failure of the veno-pressor mechanism, (45), (46), but here, too, the relative effects of changes in the arterioles and capillaries must be taken into account.

Note that in the better reactions the curve of nutrient-flow parallels more closely the curve of volume-flow.

Between observations N and O in experiment 48, the mean blood pressure increased 6 mm. Hg., the volume-flow increased from 76 to 86; the nutrient-flow, however, decreased from 36.5 to 35.5. With further injection blood pressure measurements undoubtedly would have pointed to an improved circulation though damage was possibly occurring. The observations point to a limitation of the beneficial effects of acacia injection.

The condition of lowered blood pressure is a critical one and calls for radical treatment. The blood volume should be increased by transfusion of blood or blood substitutes. Gum acacia theoretically should and practically does give beneficial effects. As Bayliss (47) showed, it has many of the desirable properties of blood and is inert and "perfectly innocuous." Even after large injections of concentrated suspensions Meek and Gasser (48) and Gasser, Meek and Erlanger (49) find no harmful effects in normal animals. If that is true methods should be available for pushing the treatment to the limit of its beneficial effects especially when blood is not available.

It should be borne in mind in pushing the injection that in addition to the over-dilution there is the danger of transudation augmented by elevated pressures. Herein lies the great difficulty of treatment in serious cases of shock and of hemorrhage going into shock, where transudation is free.

DISCUSSION

Nature of shock. The preceding data show the reactions common to hemorrhage and tissue-abuse,—the initial rise of pressure, the marked vasoconstriction, the relation of volume-flow of blood to blood-pressure, the disproportionate decrease in volume-flow to decrease in blood volume, the decrease in alkaline reserve, the normal response of the vasomotor and respiratory centers and the response of volume-flow to rise of pressure with acacia injection. In addition we may have dehydration of certain tissues, the symptoms of thirst, an increased peripheral red count, etc., in both hemorrhage and shock. (The similarity of the two conditions in connection with the increased sugar and adrenin content of the blood has been more recently brought into question by Stewart).

There are, however, some conspicuous points of difference. In hemorrhage the accessory initiating factors doing tissue damage and producing additional nutritional disturbances may be largely wanting. The condition is primarily a general disturbance resulting from a decreased blood volume and volume-flow accompanied by a dilution of blood which is a response of normal tissue. The condition of shock, however, may begin as a local or general disturbance permitting concentration of the blood from transudation and resulting in a decreased blood volume and volume-flow. In hemorrhage the ability to recover lost blood volume is little affected. In the early stages of shock this may likewise be the case as was shown by Cobbett and Roy (50) and by Vale (51). Though the intestines may increase in volume from transudation during exposure the percentage of corpuscles remains constant. The loss of plasma to the intestines shown by the decreased specific gravity of the intestines is made good by the fluids from other tissues as is indicated by the increased specific gravity of the muscle. The accessions of fluid from the more normal tissues, however, soon fail to keep pace with the fluid lost and concentration occurs. Concentration of the blood in accordance with these findings represents a local rather than a general condition and may over-emphasize the difference between hemorrhage and shock; yet Vale who confirms the results of Cobbett occasionally finds that the decreased specific gravity may occur under special circumstances in all the tissues. Gasser and Erlanger conclude from a study of blood colloids that in aortic shock dilution of the blood may occur in some parts of the body while concentration from transudation occurs in other parts. It would seem that the extent to which concentration is due to a local or general condition varies considerably and may be a matter of significance both concerning the nature and treatment of shock. One is reminded again of experiment 100 in which the local inflicted injury was insufficient to account for concentration from local transudation.

The greater severity of shock may be attributed first to the fact that shock is a result of tissue damage while in hemorrhage tissue damage may occur only from prolonged anemia. In shock the deteriorated tissue withstands more poorly a reduction in volume-flow than does the more normal tissue in hemorrhage. In hemorrhage the volume-flow of blood is improved by dilution of the blood; in shock the organism, on account of increased permeability, cannot take advantage of the favorable relation of blood volume to volume-flow. While in hemorrhage a limit is set to the transfer of fluids from the tissues to the blood by the beneficial effects derived therefrom, no limit is set in shock and the drain and transfer of fluid continues until the mechanism breaks down.

It may be that in prolonged hemorrhage, however, these points of difference disappear, the tissues suffer damage, the maintenance of blood volume is affected and the two conditions merge. In that case, disregarding other effects of tissue-abuse, the difference between hemorrhage and shock is one more of sequence of processes than of nature of processes.

There is considerable difficulty in bringing into proper relation the effects of injury and hemorrhage in conditions of lowered blood pressure, particularly at the front where the two seldom occur alone, but together in varying proportions. Surgeons recognize hemorrhage to be the most serious shock-producing factor; yet attempts are made to determine the relative importance of shock and of hemorrhage. One finds this statement for example, "It seems reasonable to conclude that the cause of death, while in part hemorrhage, was largely shock. The total amount of blood lost did not in itself appear to be sufficient to cause death." While a partial differentiation may be made in some cases yet if the factor of disturbed circulation is of importance, difficulties will be met for it may be a matter of indifference to the tissues in general whether the volume-flow is diminished as a result of external hemorrhage or of internal hemorrhage and transudation. Recall, e.g., experiment 100 cited on page 478.

The relative dangers of hemorrhage or any other form of tissue-abuse vary with the condition of the individual. Looking at shock in its broadest way from the observations of Fraser and Cowell (52) it would seem that shock begins when the individual moves from the resting quarters to the fighting area where the strain is intense. This is borne out by the findings of Robertson (53) on the effects of bleeding on the donor. "Even a slightly wounded man straight from the trenches withstands the loss of blood poorly compared with a man (convalescing in the rear) with a sprained ankle due, I believe, to the shock of battle."

When we bear in mind the concentration of the blood and the deteriorated condition of the tissues resulting from exertion, the inflicted injury, hemorrhage of varying degrees, exposure, etc., the prevalence of shock at the front is comprehensible. The more frequent occurrence of shock in the routed than in the victorious army is interesting in this connection.

Though shock is looked upon in this general way it may appear that the factors of decreased blood volume and volume-flow of blood have been over-emphasized. It is realized that various forms of tissue-abuse cannot help but have disturbing effects upon tissue nutrition for without these effects an increased permeability, transudation and decreased volume-flow could never result. And it might be stated here that the observations on the importance of pain as a shock-producing factor, the central influence over processes in the periphery, the liberation of toxins in the injured part, etc., have not been disregarded. In limiting the work to volume and nutrient-flow I do not feel that the point of view of shock has been narrowed for all the normal processes of nutrition are dependent upon an adequate volume-flow of blood. That various forms of tissue-abuse and tissue deterioration are best combatted by a free circulation appears well supported by the universal recognition by surgeons of the danger of hemorrhage.

Shock is, therefore, looked upon as a general deterioration of the tissues resulting from tissue-abuse. The misleading nature of the term shock as originally used is well recognized and is due to the emphasis on a disturbance of a single system in the organism (54). The picture of secondary shock, however, is a general one of disturbed circulation and nutrition arising from many causes more limited and local in some cases than in others. Since in every case the initial disturbance is tissue-abuse of some form leading to a general slowing of the circulation, the term *traumatic-hypodromia* appears general enough to meet all conditions and specific enough to be of some descriptive value. Since shock cannot be defined by blood pressure levels it has the advantage of applying to all stages of shock whether the pressure be normal, above or below normal.

Treatment. It is probable that the processes set up by manipulation of the intestines, by crushing of muscle, extensive burns and interference with the blood supply, etc., will present differences and yet certain essentials such as decrease in blood volume with its effects on volume-flow will be common to all. There will then be at least one common treatment, the proper increase of blood volume.

By a proper increase in blood-volume is meant one which will *a*, combat increased permeability; *b*, insure a supply of fluid to the dehydrated tissues; *c*, elicit an increased volume-flow to overcome the stasis and *d*, improve the nutrient-flow to overcome the nutritional disturbance.

a. The permeability may be combatted indirectly by improved nutrition of the tissues and directly by proper attention to the physical and chemical properties of the injected fluid. This is a problem in itself and was not made a point of special study.

b. In many cases of shock, particularly where there is a marked local transudation, dehydration of tissues may be even more pronounced than in hemorrhage. In hemorrhage all the fluid must be supplied from the exterior. In shock without hemorrhage a redistribution of the fluid by the intravenous injection of hypertonic solutions producing *internal transfusion* is a possibility. The other method consists in supplying fluid which will saturate all the tissues leaving the locally damaged tissue over-hydrated. The choice of method of increasing blood volume and supplying tissue fluid would seem to depend on the relative dangers of dehydration and over-hydration, on the relative importance of local and general transudation (55), (56), (57), (58).

c and d. In this research only the effects of external transfusion are dealt with in their relation to volume- and nutrient-flow. It might be noted that insofar as internal transfusion produces a dilution of the red cells and of the blood colloids the effects which it produces on the nutrient flow as regards some constituents, at least, are comparable to the effects of inert injection. But the suggestion of Erlanger and Gasser that hypertonic injection attracts a well balanced salt solution into the circulation should be noted.

The results on nutrient- and volume-flow obtained in this research were discussed before. Here we are interested in their application. The nutrient-flow is probably most improved by transfusion of whole blood, but it may likewise be increased several hundred per cent by injection of inert solution. The question, therefore, arises which of the methods of external transfusion should be used to combat conditions of lowered blood pressure.

The answer to this question depends largely upon the severity of the case, upon whether a nutrient-flow below normal will suffice, and on the relative importance of various nutrients and their minimum concentration compatible with normal nutrition.

The disturbances of oxidation noted point to the prime importance of oxygen, yet a lack of other nutrients might also lead to acid products of incomplete oxidation. The experiments of Abel, Rowntree and Turner (59) in which repeated hemorrhage was produced, returning only the corpuscles suspended in Locke's solution, show that relatively little plasma is essential to life. The equally good results of transfusing red cells and whole blood in man also point to the red cells as the important factor (60). Rous and Wilson (61), on the other hand, substituting horse plasma for whole blood in the rabbit, show the very small amount of hemoglobin absolutely essential to life. Mann (62) obtains best results in the treatment of shock with the transfusion of serum.

In rabbits reductions of hemoglobin to 20 per cent are survived. Much lower figures in man are on record. At the front experience calls for transfusion of blood when the hemoglobin has fallen to 20 or 30 per cent, 30 or 40 per cent is still grave but percentages above 40 per cent are no longer serious (63), agreeing with the results of Bayliss (64) who finds a loss of blood of 50 per cent to be combatted with acacia, more serious hemorrhages requiring transfusion of blood

At the Fourth Inter-Allied Surgical Conference, March 11 to 16, 1918, transfusion of blood was recommended as the method of choice in the treatment of hemorrhage. In view of the improvement and the safety of transfusion of blood and the fact that whole blood increases the nutrient-flow to a maximum, critical cases at least call for such treatment but it seems that injection of acacia has a definite place in the treatment of less serious conditions. Such injections undoubtedly would be of great benefit in cases with high pressure and moderate hemorrhage,—hemorrhage, however, which along with other forms of tissue-abuse, is sufficient to initiate the condition of shock. Administration of such preventive or rather early treatment requires the early recognition of circulatory disturbances. If acacia treatment is pushed to the limit in serious cases where blood in unavailable special precautions would also be required to avoid over-injection.

As regards shock the Inter-Allied Conference felt that indications for transfusion of blood were not as well defined as in hemorrhage. In hemorrhage, if the tissues are sound, tissue damage and loss of maintenance of blood volume result only after prolonged decreased flow and therefore if the condition is combatted early good results are to be expected. In shock the processes may be reversed, the decreased flow coming on as a result of tissue damage. The dangers are less apparent and probably more serious. Though robust individuals may be more susceptible to the development of shock, (Cobbett and Erlanger and Gasser) the severity of many cases is probably due to the reduction of the factor of safety to the minimum when the disturbance is treated. That the factor of safety is variable even in apparently healthy individuals is indicated in figures 3 and 4. Note for example the relatively great resistance displayed by dogs 87 and 103. Though the volume-flow of blood is markedly reduced the oxidations as indicated by the horizontal curve of CO_2 content are little affected. When the factor of safety is consumed, however, the break is sudden and serious as indicated by the deflection of the respiratory and CO_2 gradients. The slight resistance of dogs 85 and 102 is indicated by the prompt deflection of the gradients at the outset of the experiments.

The earlier stages and the less serious cases of shock should show a more uniform response and might well be treated with various forms of gum acacia injection so as to restore the original volume of blood. If no hemorrhage occurred, but rather concentration of the blood, the corpuscular element, provided stasis is not marked, might not suffer dilution below the normal. This may also hold to some extent for the blood colloids for Gasser and Erlanger, and Lazarus-Barlow found the concentration of these colloids to increase with transudation. Therefore, after removing the initiating factors of shock the increased volume- and nutrient-flow may in themselves lead to recovery by improving nutrition and checking further transudation directly and indirectly.

But shock with hemorrhage and serious cases of shock in which the capacity of the vascular system has increased might respond better with blood transfusion or at least a mixture of blood and acacia suspension. The dilution of blood from acacia injections requisite to produce the normal volume-flow would probably lower the nutrient-flow below that compatible with recovery. Whether the failures of treatment of shock by transfusion of whole blood are due to transfusions inadequate to maintain increased volume-flow for a long enough period, or to the irreversibility of vital processes should be determined by the employment of larger transfusions.

The suggestions derived from this work in regard to treatment are *a,* The importance of recognizing the relative dangers of various forms of tissue-abuse,—the initiating factors; *b,* The importance of recognizing the condition of shock in its earliest stages before the mean blood pressure has fallen and serious damage has been done; *c,* The need of developing methods of transfusion insuring the maximum nutrient-flow compatible with the conditions prevailing; *d,* The desirability of developing special methods, other than improved nutrient flow, to combat the permeability of the peripheral vessels.

(Among the initiating factors should be included the factors which come into play before as well as during and after the operation, for example discomfort, hunger, thirst and dehydration of the tissues from purge entailed in the not uncommon preparation of the individual for operation. The dangers of preoperative dehydration has been recognized by Cobbett, and Lazarus-Barlow has shown that saturation of the tissues with water tends to prevent concentration of the blood. The enormous effects of a small concentration of blood upon the volume-flow shown in this paper indicates the advisability of preoperative hydration rather than dehydration of the tissues.)

SUMMARY AND CONCLUSIONS

The object of this and the preceding research is to learn more nearly the nature of shock, to determine how closely normal cell nutrition is dependent upon normal volume-flow of blood and to study the factors controlling volume-flow so that this may be more exactly controlled in the treatment of conditions of lowered blood pressure.

On account of the similarities of hemorrhage and shock, the frequent synchronous occurrence of these conditions and the recognition of hemorrhage as "the most potent shock producing factor," this object seemed best attainable by a comparative study of the two conditions.

With a variety of methods employed a close dependence of normal nutrition upon normal flow of blood was indicated.

The curves of basal-flow of blood plotted against mean blood pressure are similar in hemorrhage and tissue-abuse. They show:

a. That a reduction of volume-flow amounting to 85 per cent of the initial flow may occur with a constant head of pressure or with a small change in head of pressure- a rise as well as a fall.

b. That this sudden decrease in volume-flow may after a time be as suddenly checked; with a further fall in pressure the flow may for a time remain constant, decrease or even increase.

c. That the initial decrease in volume-flow far exceeds that occurring during the subsequent fall in pressure to zero.

The similarity of the curves of basal-flow of blood in hemorrhage and tissue-abuse suggests similar vascular reactions of similar origin,– a change in caliber of the vessels being the most important factor underlying the deflection of the curves.

A decrease in blood volume of less than 10 per cent produced by hemorrhage may elicit through constriction of central origin a decreased flow of blood through the submaxillary gland of more than 60 per cent even though accompanied by a rise in pressure.

Tissue-abuse produced by manipulation of the intestines may produce a diminution in volume-flow equally as great, often accompanied by a rise in pressure.

Concentration of blood is sufficient to explain this vasoconstriction, but its effects are probably augmented by a shunting of blood into a directly abused area.

The dilatation following constriction in both hemorrhage and tissue-abuse appears attributable mainly to deterioration of the vasomotor center.

The superbasal-flow of blood in tissue-abuse as well as in hemorrhage decreases out of all proportion to the decrease in mean blood pressure.

This decrease is attributable to the increased constrictor tonus which must be inhibited and to the decreasing stretching and driving head of pressure which takes advantage of the inhibition.

The changes in basal- and super-basal flow along with the close dependence of normal nutrition on normal flow of blood explain the changes in alkaline reserve in hemorrhage and in tissue-abuse.

The relation of alkaline reserve to volume-flow of blood was considered.

The respiratory exchange in experiments on acid injection and on hemorrhage when plotted against carbon dioxide capacity was represented by a straight line. This presumably indicates a normal response of the respiratory center to decreasing alkaline reserve.

In tissue-abuse variations of this response occurred and are attributed at present not to an abnormal reaction of the center to chemical stimulation, but rather to a varying rôle played by pain reflexes.

The curves of basal-flow of blood in hemorrhage may be duplicated in the reverse direction by injection of gum acacia and then reduplicated by a second hemorrhage.

In tissue-abuse the response appears to be similar. After tissue damage occurs as a result of prolongation of the decreased volume-flow, the normal reaction of volume-flow to intravenous injection disappears.

By taking into account the factor of volume-flow times concentration of blood, as measured by the red blood cells, a basis for treatment by injection of inert solution may be worked out.

This factor designated as *nutrient-flow* may be increased several hundred per cent by inert injection.

This increase can be attributed only to the fact that the volume-flow is increased out of all proportion to the dilution of the blood eliciting the increased flow. It is in agreement with the observation that a decrease in volume-flow is out of proportion to the decrease in blood volume eliciting the decreased flow. It is explained by an application of Poiseuille's law of flow of fluids through capillary tubes.

If the effects of decreased flow are prolonged the capacity of the vascular system increases to such an extent that dilution by injection now approximates more closely the increased volume-flow, thereby retarding the increase in nutrient-flow.

Despite an increased volume-flow and an increased mean blood pressure, elicited by injection a decrease in nutrient-flow may occur.

Such conditions seem to call for transfusion of blood or at least a mixture of blood and acacia.

The conditions developing in hemorrhage and shock are shown to be same in many essentials.

Hemorrhage if left untreated may merge into the picture of shock. There are, however, important differences of which sequence rather than nature of processes and the disturbed function of maintenance of normal blood volume appear the most significant.

Since the combatting of various forms of tissue abuse on the part of the animal depends to a large extent upon an adequate volume-flow, the effects of even a slight hemorrhage illustrate why it is recognized as the most serious occurrence for the development of shock.

Shock is looked upon in a very general way as a combined circulatory and nutritional disturbance mainly of peripheral origin. The condition is initiated by a variety of forms of tissue-abuse producing tissue damage, transudation of plasma and stasis of blood. The decreased blood volume eliciting a vascular constriction reduces the flow of blood to a level below that essential for normal nutrition and far below that essential for deteriorated tissues. The condition is thus sustained by the disturbed nutrition and by the consequent inability on the part of the animal to recover the normal permeability of the vessels and therefore restore the normal blood volume that is so essential for an adequate flow of blood.

Since the circulatory and nutritional disturbances are so closely interdependent, one disturbance may become as important as the other.

Of all the numerous vicious cycles the interdependence of volume-flow and blood volume with its consequent effects on nutrition and transudation appears the most serious.

Whether the disturbed function of maintenance of blood volume requires specific treatment or is amenable to treatment by improving the volume and nutrient-flow with the transfusion of blood or acacia as employed in this research was not studied.

If improvement of the nutrient-flow is an effective measure of combatting transudation it appears that similar principles of treatment with certain modifications can be applied to hemorrhage and to shock.

The suggestions on treatment of shock derived from this work are:

a. The necessity of recognizing more fully the major forms of tissue-abuse so that shock may be more frequently prevented.

b. The need of early detection and treatment of circulatory disturbances before serious damage has been done.

c. The need for the development of methods to guide in the amount and nature of transfusion.

d. The desirability of directly as well as indirectly combatting the increased permeability of the peripheral vascular system..

BIBLIOGRAPHY

(1) COBBETT: Allbutt's System of Medicine, New York, 1897, 320.
(2) LAZARUS-BARLOW: Manual of General Pathology, Philadelphia, 1898.
(3) CANNON, COWELL, FRASER AND HOOPER: Journ. Amer. Med. Assoc., 1918, lxx.
(4) ERLANGER, GESELL, GASSER AND ELLIOTT: Journ. Amer. Med. Assoc., 1917, lxix, 2009.
(5) LUSK: The Science of Nutrition, Philadelphia, 1917, 3rd ed.
(6) ARCHIBALD AND MCLEAN: Trans. Amer. Surg. Assoc., 1917, xxxv, 522.
(7) COWELL: Journ. Amer. Med. Assoc., 1918, lxx, 607.
(8) SMITH: Brit. Med. Journ., 1918, 427.
(9) GESELL: This Journal, 1919, xlvii, 438.
(10) VAN SLYKE: Journ. Biol. Chem., 1917, xxx, 389.
(11) MILLROY: Journ. Physiol., 1917, li, 259.
(12) GESELL: loc. cit.
(13) PILCHER AND SOLLMANN: This Journal, 1914, xxxv, 59.
(14) SHERRINGTON AND COPEMAN: Journ. Physiol., 1893, xiv, 52.
(15) COBBETT: Allbutt's System of Medicine, New York, 1897.
(16) LAZARUS-BARLOW: Manual of General Pathology, Philadelphia, 1898.
(17) VALE: Med. Rec., 1904.
(18) GASSER, MEEK AND ERLANGER: This Journal, 1918, xlv, 547.
(19) MEEK AND GASSER: This Journal, 1918, xlv, 547.
(20) PORTER: This Journal, 1907, xx, 399.
(21) SEELIG AND LYON: Surg., Gynec. and Obstet., 1910, xi, 146.
(22) SEELIG AND LYON: Journ. Amer. Med. Assoc., 1909, xlv.
(23) SEELIG AND JOSEPH: Proc. Soc. Exper. Biol. and Med., 1914, xii, 49.
(24) MANN: Bull. Johns Hopkins Hosp., 1914, 205.
(25) MORRISON AND HOOKER: This Journal, 1915, xxxvii, 93.
(26) ERLANGER, GESELL, GASSER AND ELLIOTT: Journ. Amer. Med. Assoc., 1917, lxix, 2009.
(27) EPSTEIN: Amer. Journ. Sci., 1917, xxxi, 11.

(28) GESELL: Loc. cit.
(29) GESELL: Loc. cit.
(30) HENDERSON AND HAGGARD: Journ. Biol. Chem., 1918, xxxiii, 333.
(31) SCOTT: This Journal, 1918, xlvii, 43.
(32) BAYLISS: Brit. Med. Journ., 1918, 553.
(33) MANN: Bull. Johns Hopkins Hosp., 1914, 205.
(34) VAN SLYKE: Journ. Biol. Chem., 1917, xxx, 70.
(35) CANNON: Journ. Amer. Med. Assoc., 1918, lxx, 611.
(36) WRIGHT AND COLEBROOK: Lancet, 1918, xciv, 763.
(37) ROBERTSON: Brit. Med. Journ., 1918, 691.
(38) SANSUM AND WOODYATT: Journ. Biol. Chem., 1917, xxx, 155.
(39) ERLANGER AND WOODYATT: Journ. Amer. Med. Assoc., 1917, lxix, 1410.
(40) WILDER AND SANSUM: Arch. Int. Med., 1917, 311.
(41) KAUSCH: Deutsch. Med. Wochenschr., 1911, xxxvii, 8.
(42) LITCHFIELD: Journ. Amer. Med. Assoc., 1914, lxiii, 307.
(43) LITCHFIELD: Journ. Amer. Med. Assoc., 1918, lxxi, 503.
(44) MANN: Journ. Amer. Med. Assoc., 1918, lxxi, 1184.
(45) HENDERSON AND HARVEY: This Journal, 1918, xlvi, 533.
(46) HOOKER: This Journal, 1918, xlvi, 591.
(47) BAYLISS: Proc. Royal Soc., 1915–17, B, 89; Journ. Physiol., 1918, lii; Brit. Med. Journ., 1918, 553.
(48) MEEK AND GASSER: This Journal, 1918, xlv, 547.
(49) GASSER, MEEK AND ERLANGER: This Journal, 1918, xlv, 547.
(50) COBBETT: Loc. cit.
(51) VALE: Med. Rec., 1904.
(52) FRASER AND COWELL: Journ. Amer. Med. Assoc., 1918, lxx, 520.
(53) ROBERTSON: Loc. cit.
(54) LE DRAN: Reference Vale, Loc. cit., 737.
(55) SHERRINGTON AND COPEMAN: Journ. Physiol., 1893, xiv, 52.
(56) COBBETT: Loc. cit.
(57) LAZARUS-BARLOW: Manual of General Pathology, Philadelphia, 1898
(58) ERLANGER AND WOODYATT: Journ. Amer. Med. Assoc., 1917, lxix, 1410
(59) ABEL, ROWNTREE AND TURNER: Journ. Pharm. Exper. Therap 1914, v, 625.
(60) ROBERTSON: Loc. cit.
(61) ROUS AND WILSON: Journ. Amer. Med. Assoc., 1918, lxxi, 219.
(62) MANN: Journ. Amer. Med. Assoc., 1918, lxxi, 1184.
(63) FULLERTON: Lancet, 1917, i, 715.
(64) BAYLISS: Journ. Physiol., 1918, liii, xvii.

STUDIES ON THE SUBMAXILLARY GLAND

V. AN AUTOMATICALLY FILLING AND RECORDING SPIROMETER

ROBERT GESELL

*From the Physiological Laboratory of Washington University School of Medicine,
St. Louis*

Received for publication November 1, 1918

The disturbance of normal oxidations noted in conditions of lowered blood pressure may be ascribed in large part to the decreased volume-flow of blood, but the problem of oxidation is of such importance that it calls for study from various angles. By following the alkaline reserve we get an indication of the completeness of the oxidative processes and find that with decreasing volume-flow of blood there is a progressively increasing rate of neutralization of the alkaline reserve. As the alkaline reserve of the blood diminishes, two things may happen: an increased hydrogen ion concentration in the blood or an increased ventilation of the blood flowing through the lungs preventing this concentration.

It was to study in a quantitative way the reaction of the animal to decreased alkaline reserve accompanying decreased volume-flow of blood in hemorrhage and tissue abuse that this method of recording respiration was devised.

Though a large Hutchinson spirometer may be used to measure either expired or inspired air over relatively long periods of time and may frequently possess certain advantages, it has the disadvantage of lending itself poorly to the graphic method of recording and consequently is not well adapted to record irregularities and changes in respiration which may occur from time to time.

The automatically recording spirometer meets these disadvantages. Its operation is simple and it is, therefore, described (see fig.1).

The device is a modified Krogh spirometer, filled to a constant level with room air during each expiration. This air, then at the disposal of the animal, is partially inspired during each succeeding inspiration. The decrease in the volume of the spirometer occurring with each inspiration is graphically recorded and by calibrating the instrument the amount of air inspired can be determined. The device operates automatically by means of a system of valves, an electrical contact and a motor.

In the spirometer used in the present research the float, *1*, has a capacity of 1000 cc. This float is pivoted at *2* and balanced by weight, *3*. It is in communication with the exterior by means of valve, *4*, which permits the passage of air only from the exterior to the interior. The spirometer also communicates with the animal through tube, *4′*, the ether bottle, *5* and the double respiratory valve, *6*.

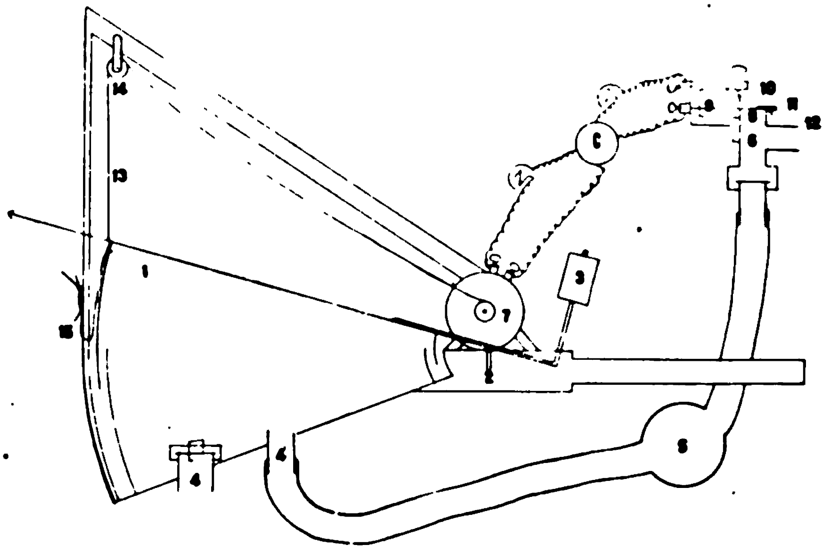


Fig. 1.

The double respiratory valve is the usual valve employed for separating expired from inspired air. It is so connected that inspired air is drawn from the spirometer and exhaled again to the exterior.

The upper or expiratory valve, *8*, made of insulating material (mica), is fitted with an electrical contact for activating the motor, *7*, which fills the spirometer with air. The valve is kept in place by means of a strip of tinsel, *9*, soldered to the platinum plate, which serves as an electrical connection. With each expiration the platinum plate is blown against the adjustable platinum contact, *10*, which closes the motor circuit and the spirometer fills.

Much depends upon the working of this valve. It is therefore described in some detail. In the ordinary respiratory valve the movements of the expiratory valve are very uncertain. If the respirations are large, the valve may be lifted bodily off its seat insuring a good contact. Very often, however, the amount of expired air is exceedingly small and the movements of the valve proportionately slight. Differences in adhesion are sufficient to make the valve open, now at one point and now at another. It was therefore desirable to devise a valve which would move freely and in the same way, with each expiration, giving a proper contact with the smaller expirations, and yet having sufficient capacity for the deepest respiration. One of the methods which has proved satisfactory is described.

The valve is weighted by a piece of lead foil, *11*, about 5 x 10 x 0.5 mm. fastened to the upper surface with Horsely's wax in the position shown. The placing of the lead foil prevents, momentarily, the rising of the valve as a whole. The lighter inner side rises to the contact, the valve turning on a hingeless joint made by placing the center of the weight over the edge, *12*.

The smallest expiration can be made effective in closing the motor circuit by adjusting contact, *10*. The opening of the valve on the contact side may with a fine adjustment be too small to allow the passage of the expired air when the respirations are increased, but when that occurs the platinum plate, blown up against the contact, forms a new pivot above and the outer edge of the valve becomes the freely moving part. It requires only a small additional increment of pressure to open this edge, the extent of this opening varying with the amplitude of respiration. The valve, therefore, adjusts itself automatically to changing magnitudes of respiration.

The operation of the apparatus requires little attention. When the electrical contact at *10* is made during expiration, the motor is activated and the float is drawn up by cord and pulley, *13* and *14*. During inspiration the motor unwinds with the descent of the float. A horizontal base line, easily adjusted to any height is obtained by checking, at a constant point, the ascent of the float, by means of a cord attached thereto.

During expiration when the float is raised the animal is protected by valve *6* against any negative pressure which may develop. Since this valve closes, room air only enters the spirometer through valve *4*. During inspiration valve *8* closes. The only air accessible to the animal is that contained in the spirometer,—this air passing

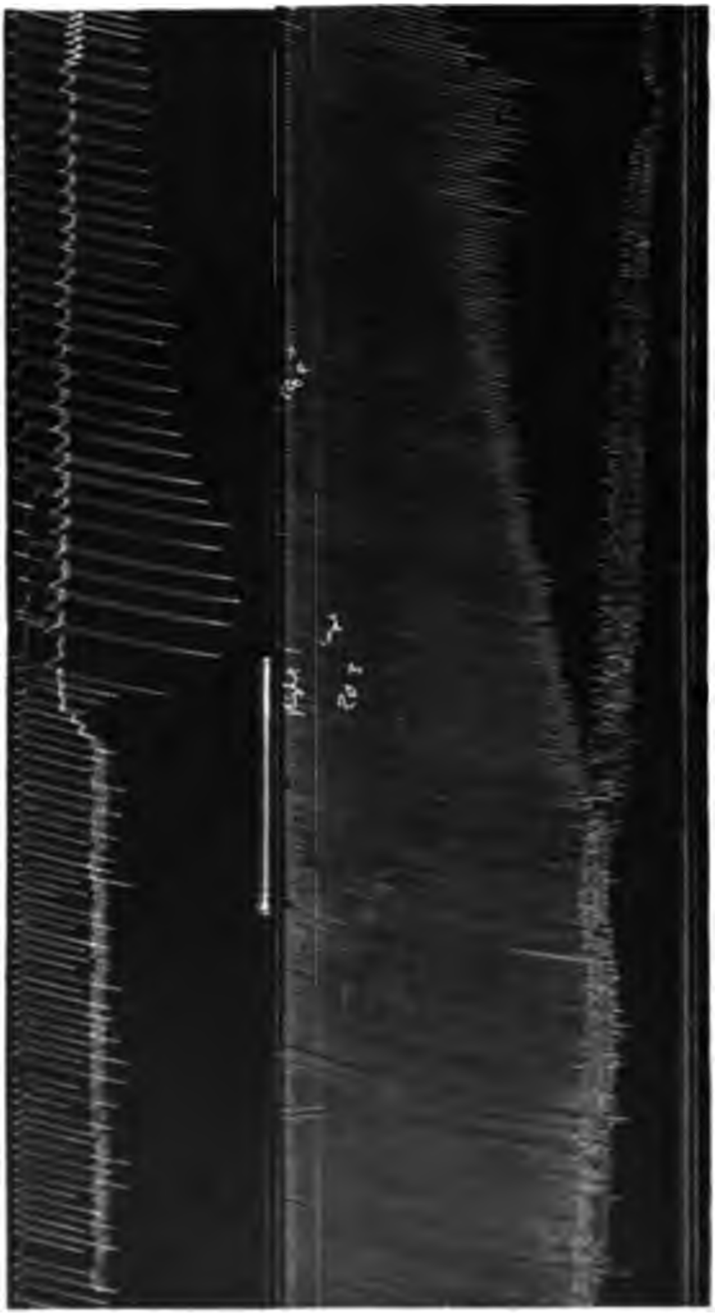


Fig. 2. Record showing changes in respiration. Upper tracing, effect of vago-section. Lower tracing—effect of lowered alkaline reserve. (Tracing taken immediately before death from acid injection.)

through valve *6*. Valves *6* and *8* are light valves the exact weight of which is of little importance for the operation of the spirometer. The weight of valve *4*, however, is of considerable importance. It must be heavy enough so that during a strong inspiration the float is drawn downward without the entrance of air through valve *4* and it must be light enough and large enough not to offer undue resistance to the entrance of air when the float is raised. The disadvantages of much resistance to the entrance of air are evident. It requires the employment of strong forces, the consumption of considerable electrical energy with the production of fling and vibration. By grading the area and thickness of the aluminum valve, *4*, and employing a minimum current in the motor these difficulties are met.

A record of respiration obtained with this device on the dog is shown in figure 2.

THE EFFECT OF OSMOTIC PRESSURE ON THE EXCITABILITY OF THE NERVE

RINNOSUKE SHŌJI

The sixth contribution to the physiology of osmotic pressure, from the Physiological Institute, Imperial University of Kyoto, Japan

Received for publication October 26, 1918

INTRODUCTION

The effect of the osmotic pressure of a solution on the excitability of a nerve, which is immersed in it, is an important part of the general physiology of osmosis. There are two hypotheses in regard to it. The one is that a hypertonic solution, which brings on a decrease of water in the nerve, causes an increase of its excitability, while a hypotonic solution, which brings on an increase of water in it, causes a decrease of its excitability. The other hypothesis states just the opposite, namely, that a hypertonic solution causes a decrease, while a hypotonic solution causes an increase of excitability of the nerve.

Eckhard (1), Kölliker (2), Birkner (3), Schiff (4) and Harless (5) observed that drying a motor nerve or withdrawing its water by means of a concentrated solution of neutral salts, urea or sugar, etc., causes an irregular excitation with clonic contractions or tetanus of the attached muscle, though both excitability and conductivity of the nerve disappeared after a certain period of time. This was confirmed by many workers. Birkner and Harless also stated that drying a nerve increased its excitability before the appearance of the clonic contractions. The latter entertained an opinion that the drying did not act as a direct stimulus to the nerve, but it caused an increased excitability shown by a muscular response to a stimulus lower than the threshold value, which stimulus, when applied to a normal nerve, gave no response at all. This hypothesis was experimentally proved by Ssubotin (6), Mandelstamm (7) and Buchner (8), while Limbourg (9) rather supported the view of Schiff, who maintained that the withdrawal of water itself acted upon the nerve as a direct stimulus. On the other hand Kölliker, Birkner, Ranke (10) and Härtl (11) proved that a gradual decrease of excitability of the nerve took place at the time when it was immersed in water or dilute NaCl solution. Ranke recognized a temporary increase of excitability in the first stage of the process. Ishikawa (12) made many experiments and proved that the hypotonic solution decreased the excitability represented by the threshold strength of electrical stimulus, and that the hypertonic solution increased it, though it gradually decreased after the solution acted for a long time.

Durig (13) noted, on the contrary, a prolongation of latent period and a diminution of the propagation velocity of excitation in the nerve of a frog which had been kept in dried air so as to diminish the quantity of water in its body. Afterwards Urano (14) expressed the opinion, based upon his own experiments, that the excitability was increased by a hypotonic solution, while it was decreased by a hypertonic solution. This was supported by Renauld (15).

Nevertheless Laugier (16) measured the threshold strength of condensor discharge, applied as a stimulus to a nerve which was immersed in a hypotonic or hypertonic solution, and found that the value of b of Hoorweg's formula, $V = \frac{a}{C} + bR$, increased, and the value of a/b decreased in both cases.

The above is an outline of the work hitherto done on the effect of osmotic pressure on the nerve.

It is interesting to determine which of these hypotheses is correct. The first step to be taken is to examine the methods and results of the experiments which led to these antagonistic hypotheses.

Harless, who is known as the originator of the first hypothesis, made his assumptions without experimental proof. As far as is known, however, Ssubotin was the first who tested this hypothesis experimentally. He immersed the nerve-trunks of nerve-muscle preparations in concentrated solutions of NaCl, $NaNO_3$, glycerol, urea and sugar, and found that, before the appearance of the clonic muscular contractions, the excitability was increased, which was represented by the threshold strength of electrical stimulus. Buchner also measured the threshold strength of electrical stimulus to estimate the excitability of a nerve immersed in saturated solutions of NaCl and urea. Härtl did the same, immersing the nerve in water. Ishikawa measured the effects of various hypotonic and hypertonic solutions, each containing the same quantity of NaCl but differing in the content of sugar, on the excitability of the nerve represented by the threshold strength of single induction shock.

The second hypothesis is based on the experiments of Urano and Renauld. Urano immersed a nerve in solutions half as concentrated, and twice as concentrated as the normal Ringer's solution alternately, and measured the threshold stimulus of a break, constant current, single induction shock and the threshold of mechanical stimulus. Renauld preferred the discharge of a condensor as stimulus, but he applied a method of an artificial circulation to the hind legs of the frog so as to test the effects of hypotonic and hypertonic solutions. The results of his experiments, however, necessarily were complicated by the effects of the solutions on the neuromuscular junctions and muscle also.

Now two of the above experiments can be taken as representatives of the two antagonistic hypotheses,—the experiment of Ishikawa for the first and that of Urano for the second.

In the present work, a series of these two experiments was repeated by the writer. Then the method of estimating the excitability of the nerve was examined and it was found that the threshold strength of electrical stimulus could not represent the excitability of a nerve in this case. The effects of hypotonic and hypertonic solutions were determined by means of measuring the propagation velocity of excitation through an affected nerve.

TEST OF URANO'S AND ISHIKAWA'S EXPERIMENTS

The methods taken in the experiments of Urano and Ishikawa agree with each other in using a nerve-muscle preparation of a frog as the material, and also in measuring the threshold strength of a break, single induction shock, to estimate the excitability of the nerve, but differ from each other in the following two points.

1. Urano used as the hypotonic solution a Ringer's solution made half as strong as the ordinary Ringer's solution, and as the hypertonic solution a Ringer's solution, made twice as strong as the ordinary one. Ishikawa, however, in all his solutions, used the same concentration of NaCl, that just sufficient to maintain the activity of the nerve, and made these solutions either hypotonic or hypertonic by adding various quantities of saccharose to them.
2. Urano immersed the nerve-trunk in his hypotonic and hypertonic solutions alternately for four hours at a time, and measured its excitability at the end of each period. Ishikawa, however, put a part of the nerve-trunk in his specially prepared vessel containing the solu-

tion, and measured the changes of its excitability with respect to time during the period of immersion.

In the present research, the solutions prepared according to Urano and Ishikawa were used separately, so as to repeat their experiments, but the latter's method was preferred to the former's, for it was then possible to better study the changes of excitability of the nerve at intervals while the solution was acting.

As the material of the experiments, a sciatic nerve of a bull-frog (Bufo vulgaris variata japonica) with gastrocnemius muscle attached was used. A part of the nerve was put in a vessel prepared according to Ishikawa. This vessel is a glass tube which has two small holes each on the opposite wall. The nerve-trunk was put through these holes and the space between the nerve and hole was plugged with vaseline to prevent the solution from leaking out. The solution was poured into the vessel. The nerve was touched by three pairs of platinum electrodes, which were alternately used. One pair (*m* in fig. 1), which were in the tube, made contact with the nerve near the side of the vessel which was opposite the muscle. This electrode was used to test the excitability of the affected part of the nerve. The other two pairs were put outside the tube; the one (*c* in fig. 1) touching the central part of the nerve and the other (*p* in fig. 1) touching the peripheral part of the nerve. The former was used to test the conductivity of the affected part of the nerve while the latter was used to test the indirect irritability of the attached muscle. When the muscle showed any change in irritability as was elicited by the last electrode, it was thrown out of the series. With respect to this method, see also Ishikawa's paper (12).

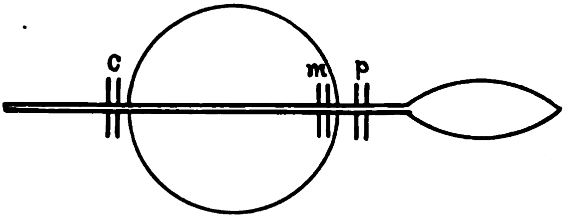


Fig. 1. A nerve-muscle preparation in vessel; *c*, *m* and *p* are platinum electrodes.

After the nerve-muscle preparation was dissected out it was preserved in Ringer's solution for one hour and then put into the vessel as prepared above. It was then allowed to remain here for two hours so as to recover from any mechanical influence on its excitability. This latter procedure was advocated by Fröhlich (17). The experiment was then commenced.

The part of the preparation outside the vessel was covered with cotton soaked with Ringer's solution. The cotton was taken off at the time of each measurement. The whole apparatus was put in a moist chamber.

When measuring the excitability, the solution was removed and all the drops of the solution attached around the nerve and the electrodes were wiped off with a hair-pencil, so that a short circuit might not occur.

The stimulating current was supplied by an induction coil. The primary was connected in series with an accumulator, a wire-resistance and a platinum-mercury key. The secondary coil was connected with any one of the three pairs of electrodes of the vessel by means of Pohl's commutators without cross wires. A break induction shock was applied to the nerve in the descending direction.

The concentrations of the physiological saline and of isotonic dextrose solutions were as follows.

Ringer's solution: 0.67 gram NaCl, 0.02 gram KCl, and 0.02 gram $CaCl_2$ (dried) in 100 grams water.

Physiological NaCl solution: 0.70 gram NaCl in 100 grams water.

Isotonic dextrose solution: 4.0 grams dextrose (without water of crystallization) in 100 grams water.

All these solutions depress the freezing point equally, i.e., $\Delta = -0.414°C$. The solvent of the solutions was water distilled through a glass or tin worm.

Two kinds of hypertonic solutions were used, namely Urano's and Ishikawa's. The former has n times the concentration of saline solutes, as the physiological saline solution, and for the sake of convenience the writer calls this "n × saline solution," while the latter is prepared by dissolving n − 1 times as much dextrose as is present in the isotonic solution in the physiological NaCl solution, which the writer calls "Ishikawa's n × solution" or "NaCl + (n − 1) × dextrose solution." The osmotic pressure of the physiological solution is called "the physiological pressure."

The experiments were carried on in the two succeeding winters of 1914 to 1915 and 1915 to 1916, in a room without heat, so that the muscle-nerve preparations might keep their activity for as long a time as possible. It was found that the nerve immersed in the physiological NaCl solution kept its excitability unchanged for fifty hours at 8 to 10°C., and for more than twenty hours when at 14 to 15°C., and for a longer time when the nerve was immersed in Ringer's solution. The experiments for this paper then were never continued longer than this time at the temperature noted.

The results of the experiments show the following facts. The 2 × NaCl and 2 × Ringer's solution gradually decreased the threshold strength of the electrical stimulus, agreeing here with Urano's experiments, while the 1.3 × and 1.5 × NaCl solution caused a slight and irregular change of the threshold strength. Experiments 1 and 2, out of seven observations made, will serve as examples.

Experiment 1. *Effect of 2 × Ringer's solution. Temperature 8.8 to 10.6°C.*

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
2 × Ringer's solution	0	0	44.0
		5	42.5
		10	41.5
		20	41.0
		40	39.0
	1	0	37.5
	1	40	34.0
	2	20	33.5
	3	20	33.0
	4	0	33.0
Ringer's solution		5	34.5
		10	35.5
		20	36.0
		40	39.0
	1	10	39.5
	3	0	39.5
	7	0	42.0

On the other hand, Ishikawa's 2 × and 3 × solutions (NaCl + 1 × dextrose and NaCl + 2 × dextrose solution) surely increased the threshold strength, but after several hours gradually decreased it, both results agreeing with those of Ishikawa. Six observations were made, one of which is shown in experiment 3.

Experiment 2. Effect of 1.3 × NaCl solution. Temperature 6.2 to 7.8°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.9 per cent NaCl solution	0	0	47.5
		10	48.0
		30	47.0
	1	0	46.0
	1	50	46.0
	3	0	48.0
	5		48.0
	8		47.5
	17		47.5
Physiological NaCl solution		30	50.0
	1	30	49.0
	4		48.0

Experiment 3. Effect of NaCl + 1 × dextrose solution. Temperature 6.0 to 8.2°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
NaCl + 1 × dextrose solution	0	0	39.5
		10	42.0
		30	44.5
		50	44.5
	1	10	44.5
	1	40	43.0
	2	20	42.5
	3	30	42.5
	4	30	42.5
	5	0	43.0
	15		38.5

As the hypotonic solution both Urano and Ishikawa used diluted saline solutions, but obtained quite different results. The result of the writer's own observations, however, differed somewhat from both these observers. The ¾ NaCl solution showed no remarkable change of the threshold strength of electrical stimulus. The ½ saline solution in most cases showed an increase of the threshold strength at first and then a decrease of it; while in some cases a decrease was observed from the beginning; again in another case, no remarkable change was apparent at all. The ¼ NaCl solution increased the threshold value at first for a short period and then decreased it remarkably. Examples of these cases are to be found in the following experiments 4 and 5, taken from a series of eight observations.

Experiment 4. Effect of ½ NaCl solution. Temperature 6.8 to 9.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.35 per cent NaCl solution	0	0	48.5
		10	53.0
		30	54.5
	1	0	55.0
	1	40	54.5
	2	10	54.5
	4		53.5
	6		52.0
	10		49.5
	18		43.5
	23		40.0
Physiological NaCl solution	2		45.0

Experiment 5. Effect of ¼ NaCl solution. Temperature 7.0 to 9.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
0.18 per cent NaCl solution	0	0	45.5
		10	46.0
		20	46.0
		40	46.0
		40	48.5
	3		52.5
	4		53.5
	8		48.0
	14		43.0
	19		37.0
	23		33.5
Physiological NaCl solution		20	34.5
	1		36.5
	3		37.0
	10		41.0

But it is possible that the effects of the diluted saline solutions mentioned above are due to the inequality of ionic concentration. To avoid this, the nerve-trunk was immersed, in the following experiments, at first in an isotonic ½ saline + ½ dextrose solution for 3 to 10 hours, until an equilibrium of ionic concentration was obtained, and then it was immersed in a ½ saline solution to allow a lower osmotic pressure to affect the nerve, independently of the inequality of ionic concentration. These experiments show that there is almost no change of threshold strength of electrical stimulus in the isotonic solution, but a gradual decrease of it is seen in the ½ saline solution. In the case of an isotonic ¼ saline + ¾ dextrose solution being applied at first to the nerve and then ¼ saline solution, almost the same result is obtained as above except an initial increase of the threshold strength caused by the isotonic solution. Experiment 6 is quoted here out of seven observations made.

Experiment 6. Effect of ½ NaCl solution. Temperature 7.0 to 10.2°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)
	hours	*minutes*	
½ NaCl + ½ dextrose solution	0	0	38.5
		20	39.0
		40	39.5
	1	0	39.5
	1	20	39.0
	1	40	38.5
	2	0	39.0
	4	0	39.0
½ NaCl solution.....		20	39.0
	1	0	38.5
	1	40	38.0
	2	20	36.5
	3	20	35.5
			33.0
	13		23.0
½ NaCl + ½ dextrose solution	1		23.5
	2		32.0
	3		33.0

The results stated above make the writer skeptical as to whether or not the excitability of the nerve is really represented by the threshold strength of electrical stimulus.

CAN THE EXCITABILITY OF THE NERVE BE REPRESENTED BY THE THRESHOLD STRENGTH OF ELECTRICAL STIMULUS?

It has been the common method to estimate the excitability of nerve by measuring the threshold strength with electrical induction shocks applied as a stimulus to the nerve. The popularity of this method is due chiefly to the readiness of its treatment and the exactness of its results. But special care must be taken in adopting this method when measuring the effect of osmotic pressure on the nerve. The reason is this:

Tanemura (18) showed that the electrical resistance of a nerve immersed in a hypotonic or hypertonic solution varied widely according to the increase or decrease of its saline contents, and that such a variation of the electrical resistance caused a deviation of the threshold strength of electrical stimulus. It was also found by the writer (19) that, in the case of the isotonic sugar solution, the variation of electrical resistance of the nerve made it impossible to estimate its excitability by the threshold strength of electrical stimulus, and that the excitability, in such a case, could be represented by the threshold strength of mechanical stimulus or the propagation velocity of excitation through the affected part of the nerve. Judging from these experiments it is quite certain that the threshold strength of electrical stimulus of the nerve, which was acted on by a hypotonic or hypertonic solution, implies an error due to the variation of electrical resistance of the nerve. Therefore the threshold strength of electrical stimulus does not represent the real excitability of the nerve, that is to say, the induction current which passes through the nerve is deformed by the variation of electrical resistance of the nerve, and accordingly the stimulating factor of the current is changed.

For this reason, in studying the effect of osmotic pressure on the excitability of the nerve, a method for measuring the excitability should be adopted which will not be influenced by any variation of electrical resistance of the nerve. In the writer's former report (19) it was stated that the threshold strength of mechanical stimulus or the propagation velocity of excitation gave exact results for the excitability in the case of the isotonic sugar solution. In the present research, however, it was found that the threshold strength of mechanical stimulus showed irregular variations in many experiments, though the method was just like the former one. This might be due partly to the change of the thickness of the nerve depending on the concentration of the solutions in which the nerve was immersed. Consequently there is only one method to be used, that is, the measurement of the propagation velocity of excitation in order to estimate the excitability of the nerve affected by the change of osmotic pressure.

MEASUREMENT OF THE PROPAGATION VELOCITY OF EXCITATION THROUGH A NERVE

It is well known that the local excitation at a stimulated point and the propagation wave of excitation which passes through this point depend upon the same physico-chemical reaction in the nervous element (20). That is to say, the excitability and propagation velocity are two functions of the same reaction, and they vary parallel with each other under changing conditions. Hence it follows that the excitability of a nerve at a given point can be estimated by measuring the propagation velocity of the excitation wave which passes through that point. This is the only reliable method of estimating the excitability of a nerve affected by osmotic pressure, as has already been pointed out.

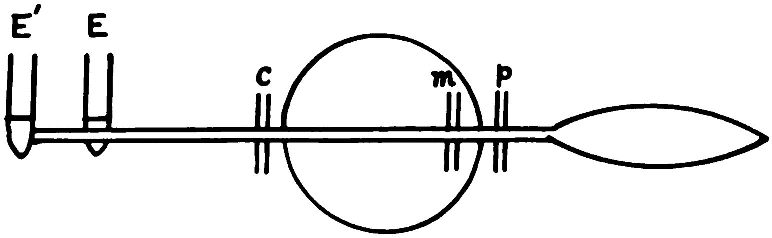


Fig. 2. The arrangement of electrodes for recording the action current; *c*, *m* and *p* are platinum electrodes. *E* and *E'* are a pair of non-polarisable electrodes, which lead off the action current to the galvanometer.

To measure the propagation velocity, the nerve is stimulated at two points, one at the central end and the other at the peripheral end of the affected part, and the velocity is calculated from the difference between the latent periods of the action current caused by the same stimulus given to each point. An Einthoven's string galvanometer is used to record the action current. The arrangement of the apparatus has already been described in a former report (19). Refer also to the figure 2 and the explanation of figure 3. A quartz string of 2300 ohm resistance was used which was drawn as tight as possible but not tight enough to produce any self-oscillation The velocity thus calculated might imply an error of 4 to 6 per cent.

In each of the various experiments, the threshold strength of electrical stimulus was also measured, for the sake of comparison, at the same time when the velocity was taken.

There is a difference in the excitability of nerve in reference to the threshold strength of electrical stimulus and the propagation velocity of excitation The latter measured by the method mentioned above is the mean velocity of all fibers of a nerve-trunk, while the former is the excitability of a few fibers, though theoretically a single fiber is desired. When a nerve-trunk is immersed in a solution, the outer layers of the trunk are affected by the solution more rapidly than the inner layers.

It was seen that, when a nerve is immersed in physiological saline solution, there is no change in the propagation velocity for fifty to sixty hours at 8 to 10°C. and for twenty-four hours at 15°C. The series of experments here reported was then made within these limits of time. The temperature was kept as low as possible, for the higher the temperature, the greater the velocity becomes, and accordingly the error of measurement increases.

The results of the experiments are as follows:

1. The effect of hypertonic solutions. At first Urano's hypertonic solution was used. When a nerve was immersed in a 2 × NaCl solution, the propagation velocity gradually decreased during the first several hours until an equilibrium was established. When the hypertonic solution was replaced by a physiological saline solution, the velocity was gradually regained. In 1.5 × and 1.75 × NaCl solution, similar results were obtained with a slight change of the velocity. Seven experiments were done with the same results, one of which is given in experiment 7.

In a 4 × NaCl solution, the velocity decreased more rapidly than in the former case and the action current became weaker and weaker until it disappeared as the nerve fibers gradually lost their conductivity. When the solution was replaced by a physiological salt solution, the nerve recovered its conductivity and the velocity of propagation was increased. In a 6 × NaCl solution, the velocity decreased still more rapidly and the action current disappeared in a shorter time. In a 7 × NaCl solution, the action current disappeared after 15 minutes. In such a short time it was impossible to examine the rate of decrease of the propagation velocity. By way of example, an experiment with 4 × NaCl solution is shown in experiment 8.

Experiment 7. Effect of 2 × NaCl solution. Temperature 7.0 to 9.0°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
2 × NaCl solution	0	49.5	16.5
	1	48.5	
	4	48.0	8.5
	5	49.0	
	6	49.0	
	8	48.5	8.2
	10	48.0	
	13	46.0	7.9
	23	41.5	7.6
Physiological NaCl solution	0.5	44.5	
	1	44.5	9.5
	2	44.5	
	4	43.5	
	5	44.5	11.9
	9	45.5	12.4
	13	46.0	13.5
	19	46.5	13.8

Experiment 8. Effect of 4 × NaCl solution. Temperature 6.5 to 8.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
4 × NaCl solution	0	0	43.5	13.2
		40	39.5	13.0
	1	5	Contractions	
	1	10	Contractions	10.4
	1	40	Contractions	
	1	50	39.0	7.0
	3	0	38.5	3.5
	5	0	35.5	—
Physiological NaCl solution	1		40.0	3.5
	3		42.0	8.1
	6		43.0	9.3
	11		43.5	11.4

In a more concentrated solution than 4 × NaCl, it was found that an excitation of the nerve occurs after the nerve is immersed a certain time. This is indicated by spontaneous clonic contractions or a tetanus. This makes the measurement of the threshold strength of electrical stimulus impossible. When these contractions occur, the galvanometer shows no action current so that the measurement of the propagation velocity is not impeded. The reason why the galvanometer shows no action current is this: The excitation of each nerve fiber occurs at a different time, so that both phases of the action currents in different fibers are neutralized by each other, thus making it impossible for the currents to be united and become of measurable intensity.

Next Ishikawa's hypertonic solution was used. In his 2 × solution (NaCl +1 × dextrose solution), the velocity began to decrease gradually from the beginning of the action of the solution, but slower than in the case of 2 × NaCl solution. When the solution was replaced by a physiological salt solution, the velocity was regained gradually. In his 1.75 × solution (NaCl + 0.75 × dextrose solution), a little decrease of the velocity was seen, while in the 1.25 × solution (NaCl + 0.25 × dextrose solution) and in the 1.5 × solution (NaCl + 0.5 × dextrose solution), its decrease was so slight that it might be regarded as an experimental error. Seven experiments were made, all with the same results, one of which is shown in experiment 9.

Experiment 9. Effect of Ishikawa's 2 × solution. Temperature 8.0 to 9.3°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
NaCl + 1 × dextrose solution	0	47.0	16.8
	1	46.5	16.2
	2	48.5	15.7
	4	50.0	14.1
	7	51.0	13.1
	12	52.0	11.8
Physiological NaCl solution	1	47.5	11.9
	3	43.0	13.1
	7	42.5	13.1
	11	43.5	14.7
	17	44.5	17.8

In Ishikawa's 4 × solution (NaCl + 3 × dextrose solution), the velocity decreased rapidly. In the 6 × solution (NaCl + 5 × dextrose solution), it decreased still more rapidly and the action current became weaker and weaker until after several hours it disappeared altogether, thus making the measurement of the velocity impossible. In the 7 × solution (NaCl + 6 × dextrose solution), the action current disappeared after an hour and at the same time clonic contractions appeared in the muscle. By way of example an experiment with 6 × solution is shown in experiment 10.

Experiment 10. Effect of Ishikawa's 6 × solution. Temperature 11.8 to 13.0°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
NaCl + 5 × dextrose solution..	0	0	49.5	18.3
		5	48.5	
		15	48.5	
		30	49.0	
		45	50.0	
	1	0	52.5	17.7
	1	20	53.5	
	1	40	54.0	
	2	0	55.5	17.1
	2	30	55.5	
	3	0	55.5	14.1
	3	30	55.5	
	4	0	56.0	10.1
	5	0	56.5	
	6	0	53.0	—
	8	0	52.5	—
Physiological NaCl solution......		5	49.5	—
	1	0	48.5	4.9
	1	30	50.0	8.9
	5	30	48.0	13.0
	10		48.0	13.2
	13		51.0	17.2

In short, the propagation velocity of excitation of the nerve gradually decreases when the nerve is immersed in any hypertonic solution. This decrease of the velocity has no relation to the variation of the threshold strength of electrical stimulus. The higher the osmotic pressure, the more rapidly the velocity decreases. The effect of a concentrated NaCl solution is stronger than that of a NaCl + dextrose solution of the same osmotic pressure. This is to be expected considering the difference of diffusion velocity of salt ions and dextrose molecules.

2. The effect of hypotonic solutions. In the ½ saline solution the propagation velocity of excitation gradually decreased, quite apart from the variation of the threshold strength of electrical stimulus. When the solution was replaced by a physiological salt solution, the velocity was, in some cases, restored to a certain degree, while in other cases it was not. Experiment 11, taken from eleven experiments, illustrates these points.

Experiment 11. Effect of ½ Ringer's solution. Temperature 9.9 to 11.0°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
½ Ringer's solution	0	37.0	17.2
	2	37.5	
	4	36.0	15.6
	8	36.5	11.1
	12	35.0	
	14	34.5	8.5
	22	33.5	7.1
Ringer's solution	2	35.5	
	4	37.0	10.7
	8	36.5	11.0
	13	38.0	11.9
	19	37.0	11.8
	32	34.5	11.8

In the ⅓ or ¼ saline solution, the velocity decreased still more rapidly than in the first case and the conductivity disappeared after a certain time. When the solution was replaced by a physiological solution, the original value for the velocity was not regained but some indications of restoration were seen by the reappearance of the velocity which had previously disappeared. Experiment 12 will serve as an example.

Experiment 12. Effect of ¼ NaCl solution. Temperature 7.0 to 8.5°C.

SOLUTION	TIME		THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours	*minutes*		
¼ NaCl solution	0	0	42.5	12.3
		20	45.5	12.2
	1		44.5	
	3		46.5	10.1
	5		50.0	8 0
	7		44.5	—
	8		44.5	—
Physiological NaCl solution		30	46.0	—
	2		42.0	6.7
	4		44.0	6 8
	8		45.5	6.1
	14		42.5	8.1
	20		44.5	7 9

Next the effect of the lower osmotic pressure was studied which did not imply a condition of inequality of ionic concentration. It was first seen that, in the isotonic ½ NaCl + ½ dextrose solution or ¼ NaCl + ¾ dextrose solution, the velocity decreased a little and then attained an equilibrium after 5 to 7 hours. The result of six experiments done confirmed this. Then, when a nerve was immersed in an isotonic ½ NaCl + ½ dextrose solution and after the equilibrium state of the velocity was attained, the solution was replaced by a ½ NaCl solution. The result was a gradual decrease of the velocity without any indication of its increase. The same procedure of experimentation was adopted in reference to another set of solutions, namely, isotonic ¼ NaCl + ¾ dextrose solution and ¼ NaCl solution, with the same result. But the decrease of the velocity in the latter series of experiments was so rapid that after five hours the nerves lost their conductivity. When the hypotonic solution was replaced by the isotonic solution, a certain degree of the velocity was restored. Experiments 13 and 14, taken from five experiments, will serve as examples.

Experiment 13. Effect of ½ NaCl solution. Temperature 14.0 to 14.5°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
½ NaCl + ½ dextrose solution	0	55.0	20.5
	2	56.5	
	4	57.0	20.0
½ NaCl solution	0.5	57.0	
	1	57.0	20.2
	2	57.0	
	4	56.5	
	9	54.0	13.3
	15	52.0	11.3
½ NaCl + ½ dextrose solution	2	55.0	
	4	57.0	11.5
Physiological NaCl solution	1	58.0	
	2	59.0	12.8

Experiment 14. Effect of ¼ NaCl solution. Temperature 14.0 to 15.8°C.

SOLUTION	TIME	THRESHOLD STRENGTH OF ELECTRICAL STIMULUS (COIL DISTANCE IN CM.)	PROPAGATION VELOCITY OF EXCITATION (M. PER SEC.)
	hours		
¼ NaCl + ¾ dextrose solution	0	46.0	21.6
	0.5	46.0	
	7	47.5	18.1
¼ NaCl solution	0.5	47.5	
	1	48.5	
	1.5	49.5	
	2	50.5	13.1
	3	50.5	
	4	52.0	6.9
	8	46.0	—
¼ NaCl + ¾ dextrose solution	1	46.0	—
	2	46.0	—
	4	44.5	10.0
	6	44.5	10.6

In a word, an osmotic pressure lower than the physiological gradually decreases the propagation velocity of excitation independent of the variation of the threshold strength of electrical stimulus. The lower the osmotic pressure, the more rapidly the velocity decreases.

To sum up the results of these experiments we can state that the propagation velocity of excitation through the nerve is at a maximum when the nerve is immersed in a solution of physiological osmotic pressure. It becomes less in accordance with a decrease or increase of the osmotic pressure below or above that of the physiological. Since the excitability of the nerve and the propagation velocity of excitation through it go parallel, the physiological osmotic pressure is the optimum pressure for the excitability of the nerve, and a lower or higher osmotic pressure than the physiological decreases its excitability in accordance with the amount of the deviation from the normal.

Fig. 3. The plate shows two kinds of experiments for measuring the propagation velocity of excitation by means of recording the action current: experiment 9 denotes the effect of a hypertonic solution (NaCl + 1 × dextrose solution), and experiment 11 denotes the effect of a hypotonic solution ($\frac{1}{2}$ Ringer's solution). Figures of the *c* series in each experiment show action currents when the stimuli are applied on the electrode *c* in figure 2. Figures of the *p* series show other action currents when the stimuli are applied on the electrode *p* in figure 2. The two white vertical lines on each figure are artificially drawn to facilitate distinguishing the moment of stimulation and the beginning of the action current. The distance between these two lines represents the time in which the action current travels from the stimulating electrodes *c* or *p* to the leading-off electrode, *E*, which runs to the galvanometer. The difference between these distances on each pair of figures of the same number represents the time of propagating the current through the nerve from the electrode *p* to the electrode *c*. The real distance from *p* to *c* is 42.5 mm., and the affected part of the nerve in the vessel is 32.5 mm. Therefore the sum of the length of the part of the nerve which lies between *p* and *c* but outside the vessel is 10.0 mm. It was assumed that the propagation velocity through this part of the nerve was unchanged through the course of the experiment, and then the propagation velocity through the affected part of the nerve was calculated. The wave line below the figure of the action current is the figure of vibration of a tuning fork, one period of which corresponds to $\frac{1}{125}$ seconds. The values of the velocity thus calculated are given in the following tables.

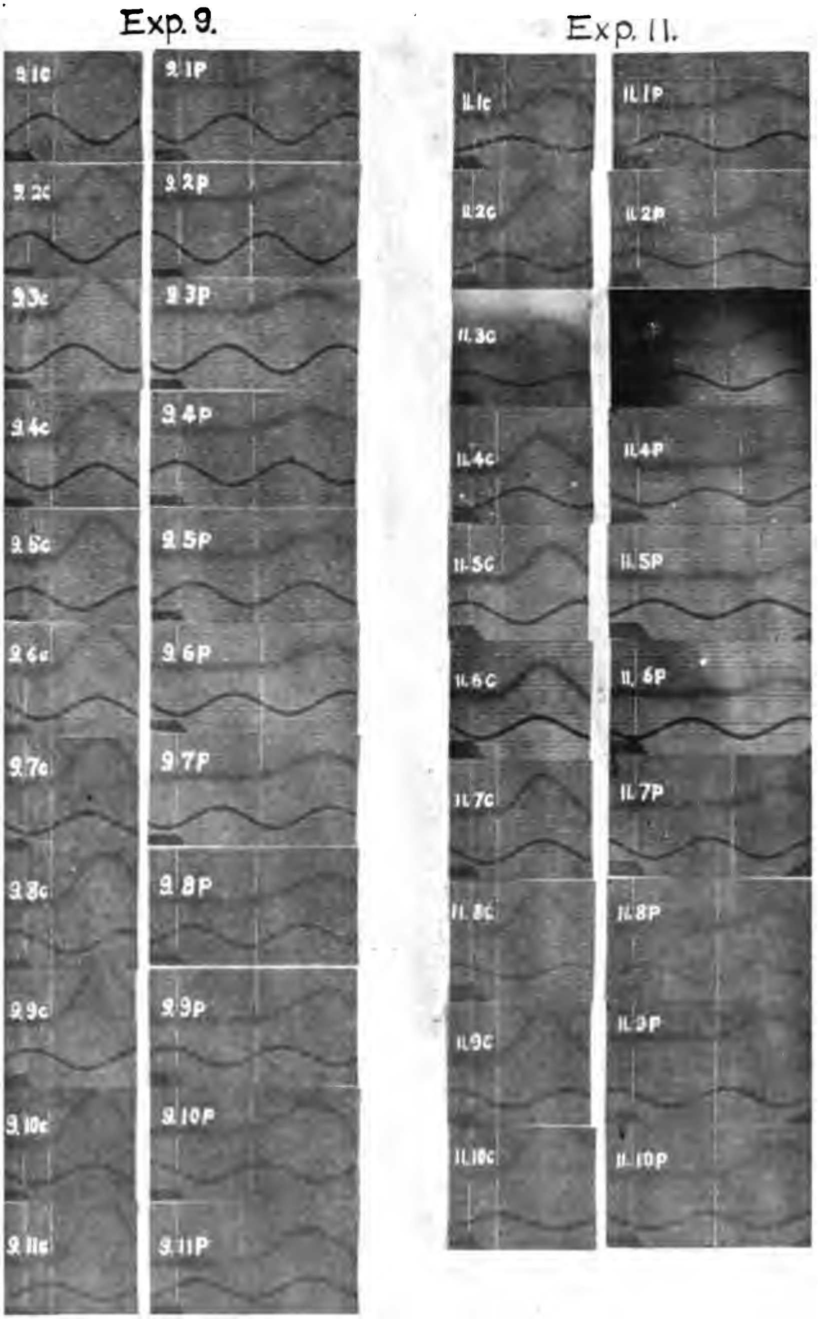

Exp. 9.
9.1c
9.1P
9.2c
9.2P
9.3c
9.3P
9.4c
9.4P
9.5c
9.5P
9.6c
9.6P
9.7c
9.7P
9.8c
9.8P
9.9c
9.9P
9.10c
9.10P
9.11c
9.11P
Exp. 11.
11.1c
11.1P
11.2c
11.2P
11.3c
11.3P
11.4c
11.4P
11.5c
11.5P
11.6c
11.6P
11.7c
11.7P
11.8c
11.8P
11.9c
11.9P
11.10c
11.10P

Fig. 3

TABLE 1

Experiment 9

SOLUTION	NUMBER OF FIGURES	TIME	TIME OF PROPAGATION BETWEEN *p* AND *c* ($\frac{1}{1000}$ SEC.)	TIME OF PROPAGATION THROUGH THE AFFECTED PART ($\frac{1}{1000}$ SEC.)	PROPAGATION VELOCITY (M SEC.)
		hours			
NaCl + 1 × dextrose solution	1	0	2.53	1.93	16.8
	2	1	2.60	2.00	16.2
	3	2	2.67	2.07	15.7
	4	4	2.91	2.31	14.1
	5	7	3.08	2.48	13.1
	6	12	3.35	2.75	11.8
Physiological NaCl solution	7	1	3.33	2.73	11.9
	8	3	3.08	2.48	13.1
	9	7	3.08	2.48	13.1
	10	11	2.82	2.22	14.7
	11	17	2.43	1.83	17.8

TABLE 2

Experiment 11

SOLUTION	NUMBER OF FIGURES	TIME	TIME OF PROPAGATION BETWEEN *p* AND *c* ($\frac{1}{1000}$ SEC.)	TIME OF PROPAGATION THROUGH THE AFFECTED PART ($\frac{1}{1000}$ SEC.)	PROPAGATION VELOCITY (M SEC.)
		hours			
½ Ringer's solution	1	0	2.47	1.89	17.2
	2	4	2.66	2.08	15.6
	3	8	3.51	2.93	11.1
	4	14	4.40	3.82	8.5
	5	22	5.16	4.58	7.1
Ringer's solution	6	4	3.63	3.05	10.7
	7	8	3.53	2.95	11.0
	8	13	3.32	2.74	11.9
	9	19	3.34	2.76	11.8
	10	32	3.34	2.76	11.8

SUMMARY

1. The change of electrical resistance of the nerve which is immersed in a hypotonic or hypertonic solution makes it impossible to estimate the excitability of the nerve by measuring the threshold strength of electrical stimulus. The excitability can only be determined by the propagation velocity of excitation through the affected part of the nerve.

2. The optimum osmotic pressure for the propagation velocity of excitation, and accordingly for the excitability of the nerve is $\Delta = -0.4$°C., which is the osmotic pressure of 0.7 per cent NaCl solution (about 5 atmospheres of pressure at 0°C.). This might well be called the physiological osmotic pressure. A higher or lower osmotic pressure than this decreases the propagation velocity of excitation, and in consequence the excitability of nerve.

In conclusion the writer wishes to express his thanks to Prof. H. Ishikawa for direction and help during the course of this research.

BIBLIOGRAPHY

(1) ECKHARDT: Zeitschr. f. rat. Med., 1851, (2) i, 303.
(2) KÖLLIKER: Würzburger Verhandl., 1856, vii, 145; Zeitschr. f. wissensch. Zoöl., 1858, ix, 417.
(3) BIRKNER: Cited by Herman, Handb. d. Physiol., ii, 1. teil, 98.
(4) SCHIFF: Lehrb. d. Muskel- u. Nervenphysiol., 1858, 101.
(5) HARLESS: Zeitschr. f. rat. Med., 1859, (3) vii, 219.
(6) SSUBOTIN: Centralbl. f. med. Wissensch., 1866, 737.
(7) MANDELSTAMM: Cited by Cremer, Nagel's Handb. d. Physiol., iv, 823; also cited by Limburg (9).
(8) BUCHNER: Zeitschr. f. Biol., 1876, xii, 129.
(9) LIMBURG: Pflüger's Arch., 1887, xli, 303.
(10) RANKE: Cited by Herman, Handb. d. Physiol., ii, 1. teil, 99.
(11) HÄRTL: Arch. f. Physiol., 1904, 91.
(12) ISHIKAWA: Zeitschr. f. allg. Physiol., 1912, xiii, 227.
(13) DURIG: Pflüger's Arch., 1901, lxxxvii, 42; 1902, xcii, 322.
(14) URANO: Zeitschr. f. Biol., 1908, l, 459.
(15) RENAULD: Arch. int. de Physiol., 1910, ix, 101.
(16) LAUGIER: Journ. d. Physiol. et d. Path. gén., 1910, xii, 26.
(17) FRÖHLICH: Zeitschr. f. allg. Physiol., 1904, iii, 148.
(18) TANEMURA: Acta schol. Med. Univ. in Kioto, 1916, i, 381.
(19) SHŌJI: Acta schol. Med. Univ. in Kioto, 1916, i, 395.
(20) VERWORN: Erregung und Lähmung, 1914, 108.

A PHYSIOLOGICAL STUDY ON THE LUMINESCENCE OF WATASENIA SCINTILLANS (BERRY)

RINNOSUKE SHŌJI

From the Physiological Institute, Imperial University of Kyoto, Japan

Received for publication, October 26, 1918

INTRODUCTION

Watasenia scintillans (Berry), a luminous cephalopod, is found along the northeastern coast of Toyama-Bay in the Japan Sea. It is commonly called "Hotaru-ika" in our national language meaning literally firefly-squid.

Up to the present time what is called Abraliopsis, a genus of luminous cephalopods, has been believed to be very rare, and in fact only a few samples have been collected. The studies of this animal were made by L. Joubin (1), W. Hoyle (2) and C. Chun (3). Several years ago S. Watase (4) reported large numbers of a species of this genus occurring in the sea mentioned above, and named it Hotaru-ika. Its morphology has since been studied independently by S. S. Berry (5), C. Ishikawa (6), M. Sasaki (7) and S. Matsuno (8). Berry named it Abraliopsis scintillans, but Ishikawa stated a few years later that it did not belong to this genus, and named it Watasenia scintillans (Berry) after the name of its discoverer.

This animal is a small squid with a mantle-length of 6 cm. Like Abraliopsis it has three kinds of luminous organs which Watase called the luminous organs of the first, second and third classes. The luminous organ of the first class, or the brachial organ, is the largest of all. It is at the end of each fourth arm and consists of three oval globules arranged in a series. The luminescence of this organ is stronger than the other two. The luminous organ of the second class, or the ocular organ, consists of five small organs which are arranged in a series along the ventral circumference of each eyeball. The luminous organ of the third class, or the cutaneous organ, consists of numerous minute organs dotted about on the ventral surface of the whole body. The microscopical structure of these luminous organs is nearly the same as that of Abraliopsis morisii Vérany, which was described by Chun. Joubin and Chun were already aware of the light-producing function of the ocular and cutaneous organs, though they had not examined the living animal. The function of the brachial organ was not known until Watase observed its luminescence in the living animal.

The writer of this paper made physiological researches upon the luminescence of this animal in May and June of 1915 and 1916 at Uwozu, a town situated on the coast of Toyama-Bay.

THE EFFECT OF CONCENTRATION OF SEA WATER ON THE LIFE OF WATASENIA SCINTILLANS (BERRY)

Fishermen who catch this squid speak of it as being so delicate an animal that it lives only a very short time if it is put in a pail containing the sea water of the uppermost layer. It occurred to the writer that this might be due to a lower salinity of the uppermost layer near the coast rather than that of deep sea water. To test this idea the following experiments on the effects of concentration of the sea water on the life of this animal were made.

In order to compare the effects of sea water from various levels on the life of the animal, one squid was placed in 2 liters of sea water and left until it died. A record of the average time obtained in several experiments of the same kind was taken. Care was taken not to allow the ink spouted by the animal to become mixed with the sea water, for it is very injurious to the life of the animal.

The relation between length of life of the animal and the concentration of the sea water at various depths was first determined. By measuring the value of the osmotic pressure and the specific gravity, it was found that sea water below a depth of five meters has nearly the same concentration and has no injurious effect upon the life of the animal, while the sea water of the uppermost layer, which is relatively more dilute, shortens the animal's life. An example of experiments on this point is shown in table 1.

Next, the effects of the concentration of the sea water upon the life of the squid was estimated, using: *a*, deep sea water of 10 to 20 m., which was regarded as the standard solution; *b*, various dilutions of sea water made by adding distilled water; and *c*, various concentrations of sea water prepared by dissolving in it varying amounts of salt obtained from the deep sea water by evaporation. It was found that animals lived longest in the normal sea water, and a lesser time in diluted sea water in proportion to the degree of dilution, especially in sea water more highly diluted than $\Delta = -1.5°C$. The same result as in the diluted sea water was obtained in a concentrated sea water but the shortening was not so marked. These experiments were repeated eight times. One series is shown in table 2.

Judging from the results shown in tables 1 and 2, it is obvious that the injurious action of the surface layer of sea water is due to its dilution. But this is not the only factor, for life should be lengthened in a solution prepared by dissolving the salt obtained from the deep sea water in either fresh water or sea water of an uppermost layer making its concentration equal to that of deep sea water. But according to the writer's experiments life is remarkably longer in the latter than in the former.

TABLE 1

Relation between the depth of the sea water and the length of life of Watasenia. Experimental temperature 18.1° to 18.8°C.

DEPTH OF THE SEA WATER	SPECIFIC GRAVITY AT 15°C.	FREEZING POINT DEPRESSION	CALCULATED CONCENTRATION OF ISOTONIC NaCl SOLUTION	MEAN LENGTH OF LIFE	
meters		*degrees C.*	*per cent*	*hours*	*minutes*
Uppermost layer	1.0125	−0.839	1.44		10
5	1.0254	−1.816	3.16	2	36
15	1.0260	−1.870	3.25	3	40
45	1.0261	−1.860	3.24	1	47
90	1.0260	−1.844	3.21		58
135	1.0267	−1.900	3.31	1	14
180	1.0266	−1.885	3.28	1	55

Next it was attempted to estimate the effect of the dilution of sea water upon the osmotic pressure of the blood of Watasenia. But as this squid was too small to get a sufficient quantity of blood, some Ommastrephes were used in place of Watasenia in the following experiment. Several living Ommastrephes were placed in sea water of known osmotic pressure. After the death of the animals or after an hour in their living state, the blood was removed from the animal and its osmotic pressure was measured. The result of the measurement is as follows.

Freezing point depression of the sea water of 10 m. deep layer...−1.780°C.
Freezing point depression of the blood of the squids which are living in the above-mentioned deep sea water..............−1.796°C.
Freezing point depression of the sea water of the uppermost layer..−0.537°C.
Freezing point depression of the blood of the squids which died in the same sea water of the uppermost layer.........−1.571°C.

TABLE 2

Relation between concentration of the sea water and the length of life of Watasenia. Specific gravity of 10 m. deep sea water, 1.0256 (at 15°C.). Experimental temperature, 19.5° to 21.0°C.

SOLUTION	FREEZING POINT DEPRESSION	CALCULATED CONCENTRATION OF ISOTONIC NaCl SOLUTION	MEAN LENGTH OF LIFE	
	degrees C.	*per cent*	*hours*	*minutes*
1.0 l. sea water + 1.0 l. water	−0.918	1.58		5
1.1 l. sea water + 0.9 l. water	−1.011	1.74		7
1.2 l. sea water + 0.8 l. water	−1.100	1.90		10
1.3 l. sea water + 0.7 l. water	−1.185	2.05		14
1.4 l. sea water + 0.6 l. water	−1.277	2.21		33
1.5 l. sea water + 0.5 l. water	−1.367	2.37		32
1.6 l. sea water + 0.4 l. water	−1.458	2.53		35
1.7 l. sea water + 0.3 l. water	−1.547	2.69	1	13
1.8 l. sea water + 0.2 l. water	−1.631	2.84	1	56
1.9 l. sea water + 0.1 l. water	−1.722	3.00	3	5
2.0 l. sea water	−1.813	3.16	3	0
2.0 l. sea water + 3 grams salt	−1.898	3.31	2	24
2.0 l. sea water + 6 grams salt	−1.982	3.46	1	6
2.0 l. sea water + 9 grams salt	−2.065	3.61	1	33
2.0 l. sea water + 12 grams salt	−2.150	3.76	1	5
2.0 l. sea water + 15 grams salt	−2.234	3.91	1	13

As is well known, the blood of a living squid has the same osmotic pressure as that of the sea water in which it lives. But this experiment shows that the osmotic pressure of the blood does not decrease parallel with that of the sea water when the latter becomes very low, and the animal dies when the osmotic pressure of the blood decreases to a certain degree. Lack of sufficient material prevented further investigation of this point.

Oxygen dissolved in the sea water is of course an important factor for maintaining the life of Watasenia. Three squids were placed in a specially equipped barrel containing 25 liters of deep sea water through which oxygen gas was passed against an inner pressure of two atmospheres, so that the solubility of oxygen might be increased. Under these conditions one squid lived 18 hours; the other two 22 and 26 hours respectively. A control experiment was made under the same conditions differing only in keeping the inner pressure normal and without passing oxygen gas. In this case all three squids died after 6 hours. The results were confirmed by two other experiments of the same kind.

OBSERVATIONS OF THE LUMINESCENCE OF A LIVING WATASENIA

1. Observations of the luminescence of the luminous organs of the first class: Under natural conditions of living, the luminescence of the organs of this class is rarely seen. But as a consequence either of a mechanical or of any other stimulus, or when the sea water in which the animal is living becomes unsuitable for its life, the organs begin to function. The emission of light is periodical as in the case of the firefly; the duration time of one emission is not longer than 30 seconds and the interval varies considerably with varying conditions. Organs function equally well by day or by night. The light is bluish-white and shows a continuous spectrum, the extent of which could not be measured because of the type of spectroscope then available.

Upon the cessation of respiration or when the fourth arm is removed, this luminescent organ can no longer function. The following fruitless experiments were made to test this particular point: First the application of an electrical or mechanical stimulus directly upon the organ, indirectly upon the cut end of the arm, and then upon the pair of ganglia which are situated on the back of the head and are special to Watasenia. (This ganglion was detected by Prof. C. Ishikawa, and the writer is indebted to him for the information.) Stimuli applied to other parts of the body also gave negative results.

After the death of the animal the luminous organ is covered with pigment layers, but this can not be considered as the reason of the absence of luminescence for when the pigment cells contract so as to reveal the luminous substance, or when they are artificially removed, luminescence is no longer apparent.

2. Observations of the luminescence of the luminous organs of the second class: Observation of these organs which are situated on the ventral side of the eyeball is somewhat difficult in the living animal. To observe them the animal must be fixed on its dorsal side. But in this position the animal dies in a short time. Moreover it is not easy to distinguish the luminescence of the organs of this class from that of the organs of the third class, which are scattered about in the skin over the eyeball. According to the writer's observations the luminescence of the organs of the second class is almost the same as that of the organs of the third class.

3. Observations of the luminescence of the luminous organs of the third class: Though the organs of this class are very numerous (about 600 on the mantle only), their illumination is so faint as to be just visible at a distance of one meter in a dark room. These ventrally located organs may be studied to advantage and with little disturbance to the animal by placing it over a mirror in a dish of sea water.

The intensity of the light varies from time to time and may almost disappear. Yet the luminescence cannot be said to be periodic as in the case of the organs of the first class, but is continuous. For instance, it was often observed that a single organ maintained its luminescence for more than 20 minutes. The color of the light of the organs is the same as that of the organs of the first class.

Within a short time after the death of the animal, the organs of the third class glitter with a blue light as seen by daylight, simulating the real luminescence. This condition was noticed by Joubin in the skin organs of Histioteuthis (9) and Abraliopsis. However, in the organs of Watasenia, many instances were observed in which the organs thus functioning in the daylight showed no luminescence when observed in a dark room. Again this glitter could not be seen from an oblique direction. We can infer from this phenomenon that it is not the real luminescence but perhaps only a reflection of the daylight from the organs, just as in the case of a cat's eyes which reflect in a dimly lighted room. This glitter of the organs disappears after a certain time and only numerous gray points are seen. But even in this state their luminescence can still be observed by application of a stimulus to the organs.

When a mechanical or electrical stimulus is applied to a living animal or to a dissected mantle, the organs of the third class illuminate strongly.

The dissected mantles were used to study the effects of various conditions upon the luminescence of this animal, because the organs of the first and second classes can not conveniently be used for this purpose.

ON THE LUMINOUS SUBSTANCES

As is well known, the bioluminescence is due to the oxidation of a certain substance produced by the cells of the luminous organs which are differentiated glands. According to R. Dubois (10) this oxidation is catalysed by a special oxidative enzyme produced by the organism. He extracted a luminous substance and an oxidative enzyme from the secretion of Pholas dactylus and named them luciferine and luciferase respectively. Recently E. N. Harvey (11) reported that the luciferase which he extracted from Cypridina and the firefly was not an enzyme, but the source of the light. H gave the names of photogenin for luciferase and photophelein for luciferin.

But the presence of such luminous substances cannot be experimentally proved in all kinds of luminous organisms. According to Pütter (12) the bioluminescence is of two kinds, viz., extracellular and intracellular luminescence. In the animal in which extracellular luminescence takes place, the luminous organ still maintains the original structure of a gland, having both alveoli and ducts. The secretion is in this case oxidized so as to emit light soon after it is secreted in the cavity of the alveoli or after it is expelled from the gland. From a luminous organ of this kind the luminous substance can be extracted. In the animal in which intracellular luminescence takes place, the luminous organ is highly differentiated, alevoli and ducts being reduced to a mass of cells. The luminous substance is made to produce light in the cells as soon as it is produced, so that no secretion is expelled from them. As can be easily seen from this, it is very difficult, if not impossible, to extract the luminous substance from the organ of this class.

The luminesence of Watasenia belongs to the type of intracellular luminescence because the histological structure of the luminous organs shows neither alveoli nor ducts. Therefore the extraction of the luminous substance from this animal is undoubtedly very difficult. In fact several unsuccessful attempts were made to extract it with water and several organic solvents.

NECESSITY OF OXYGEN FOR LUMINESCENCE

Since bioluminescence is an oxidative process, oxygen is absolutely necessary. The luminescence of Watasenia may be taken as an example. According to the writer's observations, a dissected mantle emits light more strongly in air than in the sea water and most strongly in pure oxygen. It was observed that when a mantle was put in free atmospheric air, its light disappeared altogether after 15 hours when its outer surface became dry, while another piece which was put in a glass vessel in order to prevent its quick drying (for this purpose a Verworn's gas chamber of about 10 cc. was used), kept its luminescence for more than 25 hours (at room temperature of 20 to 23°C.).

Again putting the third piece in a gas chamber and passing hydrogen or carbon dioxide gas through it, it was observed that the luminescence disappeared after 10 to 25 seconds. When these gases were replaced by air, the luminescence reappeared in from a few to some 20 seconds. When pure oxygen was used instead of air, the luminescence became more intense than in air. Such an experiment could be repeated with a single specimen as many times as one wished.

Consequently it may be stated that oxygen is indispensable for the luminescence of Watasenia.

THE EFFECT OF NARCOTICS ON LUMINESCENCE

It is said that in many luminous organisms narcotics first stimulate, then inhibit the bioluminescence. The effect of narcotics on Watasenia agrees with this statement. When mantles are placed in a Verworn's gas chamber and air saturated with vapor of alcohol, ether or chloroform is passed through it, the luminescence becomes more intensive at first, then gradually diminishes and after a certain time disappears. But if the narcotics are replaced by air, the luminescence reappears in a few seconds. The effect of ether is most injurious, chloroform next and alcohol least. But these differences may not be attributed to the nature of the narcotics, for the vapor pressures of these narcotics (at room temperature of 20°C., at which the experiments were made) are different, but parallel to the different intensities of their activity. The results of the experiments are shown in table 3.

TABLE 3

The effect of narcotics on the luminescence of Watasenia. Experimental temperature, 20°C.

NARCOTICS	TIME REQUIRED TO REACH THE MAXIMAL LUMINESCENCE	TIME REQUIRED FOR THE DISAPPEARANCE OF LUMINESCENCE	TIME REQUIRED FOR THE RECOVERY OF LUMINESCENCE IN AIR	SATURATED VAPOR TENSION OF NARCOTICS AT 20°C.
	seconds	*seconds*	*seconds*	*mm. Hg.*
Ether	1	2–3	1–3	442.4
Chloroform	1.5–3	6–10	1–3	160.5
Alcohol	5–20	60–90	1–3	44.0

We may conclude, therefore, that narotics first excite then inhibit luminescence and that this inhibition is reversible.

THE EFFECT OF TEMPERATURE ON LUMINESCENCE

The results of many experiments on the effect of temperature upon bioluminescence are in complete agreement on the point that there is a limited optimal range of temperature for bioluminescence and that any temperature outside this range acts unfavorably upon it. This optimal range of temperature is variable according to the materials and to the method which is employed.

TABLE 4

The effect of temperature on the luminescence of Watasenia

TEMPERATURE	DURATION OF LUMINESCENCE		RECOVERY OF LUMINESCENCE WHEN RETURNED TO 26°C.
degrees C.	*hours*	*minutes*	
−12.0		2	Recover after 10 minutes
− 3.3		5	Recover after 2 minutes
10.0		25	Recover after 3 minutes
17.0 (16.0–18.0)	12		
20.1 (18.8–21.4)	13		
21.0 (20.0–22.0)	12		
24.0 (constant)	11		
25.0 (constant)	12		
28.7 (29.3–28.0)	13		
30.4 (31.8–29.0)	10		
31.6 (32.6–30.6)	5		
32.0 (32.5–31.5)	3		
33.6 (34.0–33.2)	1		Recover after 1 minute
35.0		30	Recover after 1 minute
36.0		16	Recover after 1 minute
39.0		10	Recover after 1 minute
40.8		2	Recover after 1 minute, but irreversible after 2 or 3 repetitions
44.5		1	Recover after 3 minutes, but irreversible after 2 repetitions
48.8		1	Irreversible, mantles coagulate
63.0	Immediately		Irreversible, mantles coagulate

Experiments upon the effects of temperature on the luminescence of Watasenia were made, the results of which are shown in table 4. The following method was used. Dissected mantles were put in a test tube which was again put in a Dewar's flask (a thermos bottle) containing water at a constant temperature. The flask was closed with a cork through which a thermometer was inserted. The inner temperature varied somewhat as the time passed on. The initial and the final temperature are given parenthetically in the table.

From these results it may be concluded that the optimal range of temperature for the luminescence of Watasenia is from 16°C. to 31°C. Within this range luminescence persists through about the same time interval but the intensity of the light is stronger at the lower temperature (16 to 20°C.) than at the higher. The inhibition of luminescence at a lower temperature than 10°C. (down to −12°C.) is perfectly reversible. The inhibition at 36 to 45°C. is also reversible but after repeated experiments or when heat is applied for a long time the luminous organ loses its reversibility. A higher temperature than 49°C. entirely destroys the illuminating power of the organ due to the heat coagulation of its cells.

THE EFFECT OF SUNLIGHT ON LUMINESCENCE

Almost all work hitherto published refers to the fact that the intensity of bioluminescence is diminished after the exposure of the animal or the luminous organ to sunlight or to strong artificial light. But the results obtained by the writer differ from this.

Many dissected mantles of Watasenia were exposed to the sunlight for from 20 minutes to 2 hours, taking precautions to prevent desiccation, but no difference in luminescence was observed between the materials which were brought into a dark room after exposing to the direct sunlight for even 2 hours and the materials which were kept in the dark room from the beginning.

THE EFFECT OF OSMOTIC PRESSURE ON LUMINESCENCE

It is well known that, when the luminous organ of an animal is dried or immersed in glycerol to withdraw its water, it loses completely the illuminating power. But with regard to the effect of immersing the organ in water, various results are reported by various workers. There is no information on the effect of osmotic pressure upon the bioluminescence, except the work of Kölliker and of Mangold. Kölliker (13) observed that the 12 to 20 per cent NaCl solution intensified the luminescence of Lampyris at first, and then after a short time paralyzed it. Mangold (14) observed that a concentrated NaCl solution intensified the luminescence of Ophiopsila but not that of Maurolicus.

Watasenia is favorable for testing the effect of osmotic pressure upon bioluminescence, for it lives in the sea. Some dissected mantles were put in the sea water of various concentrations and the effects of these solutions on luminescence were examined. The solutions were prepared as follows: Salts obtained from evaporated sea water were redissolved in sea water and the concentrated sea water thus obtained was filtered. Various dilutions were obtained by adding distilled water to the concentrated sea water. Unmodified sea water was always used as a control.

TABLE 5

The effect of osmotic pressure on luminescence. Experimental temperature 16.8° to 18.8°C.

OSMOTIC PRESSURE OF SOLUTION (THE PRESSURE OF NORMAL SEA WATER BEING TAKEN AS 1)	CONCENTRATION OF ISOTONIC NaCl SOLUTION	TIME OF PERSISTENCE OF THE LUMINOUS POWER (IRRITABILITY TO THE STIMULUS)
	per cent	*hours*
3.7	11.14	1
3.2	9.74	1
2.3	6.96	1
2.0 (Δ = −3.448)	6.06	1
1.9	5.76	2
1.8	5.46	4
1.7	5.15	6
1.6	4.85	6
1.5	4.55	7
1.4	4.24	9
1.3	3.94	9
1.2	3.64	10
1.1	3.33	10
1.0	3.03	10
0.9	2.73	10
0.8	2.42	10
0.7	2.12	10
0.6	1.82	10
0.5	1.51	10
0.4	1.21	9
0.3	0.91	9
0.2	0.61	9
0.1	0.30	4
0 (water)	0	1
sea water (Δ = −1.800)	3.24	10

The dissected mantles were immersed in sea water of different concentration and maintained their luminescence for approximately half an hour. Luminescence persisted longer in dilute than in concentrated sea water. But in all cases luminescence ceased entirely after about an hour. When a mechanical stimulus (a slight rubbing) was applied to a mantle that had already lost its luminescence in the sea water, the luminous organ again illuminated as a response to the stimulus. This irritability was maintained for the longest time in the sea water which was isotonic to 0.6 to 4 per cent NaCl solution. A higher or lower osmotic pressure than this range shortens the time that this irritability will persist.

The results of experiments with NaCl solutions of different concentration were the same, though the absolute value of the persistence of irritability was shorter than in the case of the sea water.

Table 5 is an example out of six series of experiments.

THE ACTION OF IONS ON LUMINESCENCE

It is found by many workers that strong acids, alkalis or salts of heavy metals destroy the luminous power of many luminous organisms. With regard to the action of neutral alkali-metal salts, some stated their exciting action while others found them to be without effect upon bioluminescence. It must be remembered that these experiments were made mostly with luminous insects and bacteria.

Watasenia furnishes good material for studying the action of ions upon bioluminescence. The writer made several series of experiments on this point, which are described in the following three parts.

a. The action of hydrogen and hydroxyl ions. In order to examine the action of H, or OH ions, materials were immersed in the sea water containing HCl or NaOH of a concentration of 1/6400 N to 1/50 N. The reason why HCl and NaOH were chosen for this purpose was that Cl and Na ions are present in large amount in sea water. It was found that H and OH ions first excited luminescence in the squid in proportion to their concentration, and afterwards inhibited the luminescence. H ions destroyed the luminous power, i.e., the irritability to a stimulus, while OH ions had no injurious action upon it, when its concentration was less than 1/50 N.

b. The action of ions of a single compound. In order to study the action of cations and anions separately, chlorides of various metals and Na-salts of various acids were used. Isotonic solution of these salts were prepared whose osmotic pressure were equal to, or one-half or one-third of that of the sea water ($\Delta = -1.860$°C.). Isomolecular solutions were not used because of a large difference of osmotic pressure between the solutions of univalent and bivalent salts. The concentration of salts in the isotonic solutions was calculated from the formula $\frac{\text{molecular weight}}{\text{molecular freezing point depression}} \times$ freezing point depression of solution.

TABLE 6

The effect of hydrogen and hydroxyl ions on luminescence. Experimental temperature 15.0° to 18.8°C.

CONCENTRATION OF ACID OR ALKALI CONTAINED IN THE SEA WATER	TIME OF PERSISTENCE OF THE LUMINESCENCE	TIME OF PERSISTENCE OF LUMINOUS POWER	
	minutes	*hours*	*minutes*
1/50 N HCl	5		5
1/100 N HCl	5		5
1/200 N HCl	20		20
1/400 N HCl	20	5	
1/800 N HCl	40	5	
1/1600 N HCl	40	6	
1/3200 N HCl	40	5	
Pure sea water	40	6	
1/3200 N NaOH	40	6	
1/1600 N NaOH	40	5	
1/800 N NaOH	40	5	
1/400 N NaOH	40	5	
1/200 N NaOH	40	5	
1/100 N NaOH	20	6	
1/50 N NaOH	5	6	

The molecular freezing point depression of various salts at a fixed osmotic pressure was calculated from the data given in Landolt's "Physico-chemische Tabellen." As for the substances NaI, $NaHCO_3$, NaH_2PO_4, $NaNO_2$, and Na-tartrate, no data being available, the writer measured their molecular freezing point depression by the usual method.

Materials were immersed in these solutions and their luminescence was observed. Tables 7 and 8 are given as examples.

The results with acidic and basic salts, such as NH_4Cl, $NaHCO_3$, NaH_2PO_4 and NaSCN, $NaCH_3CO_2$, Na_2SO_3, Na_2HPO_4, Na-tartrate are not to be taken into account because the solutions of such salts contain H or OH ions, which act as such upon the materials, though these salts are commonly dealt with by many investigators in a lyotropic series for various physical and physico-chemical properties.

The order of a lyotropic series of ions for physical and chemical properties in acidic solutions is in many cases the reciprocal of that in alkaline solutions. So, in this experiment, the actions of ions in acidic and alkaline solutions were tested, adding 1/600 N HCl and 1/600 N NaOH respectively to them. But it was found that the results in both cases were the same and not contradictory to each other.

TABLE 7

The action of cations on luminescence. Experimental temperature 16.4° to 18.6°C.

SALT	CONCENTRATION ($\Delta = -0.93$°C.) IN PER CENT	INTENSITY OF ILLUMINATION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER	
			hours	*minutes*	*hours*	*minutes*
$LiCl+2H_2O$	2.02	+		40	4	0
NH_4Cl	1.46	−		0		0
NaCl	1.62	+		20	1	20
KCl	2.07	++		20	1	20
RbCl	3.42	−		0		0
CsCl	4.81	++		2		40
$MgCl+6H_2O$	3.58	++	1	20	3	0
$CaCl_2$(sicc.)	2.05	+		20	1	20
$SrCl_2+6H_2O$	5.00	+		20	1	20
$BaCl_2+2H_2O$	4.78	+		2		2
$MnCl_2+4H_2O$	3.62	++		20		20
$FeCl_2+4H_2O$	3.47	++		20		20
$ZnCl_2$	2.40	++		5		5
Sea water	½ dil.	+		20	4	0

Intensity of the luminescence is denoted as follows:

— denotes that the illumination immediately disappears.

+ denotes illumination.

++ denotes an intensive illumination which is seen from a distance of 80 cm

From the results of experiments it may be concluded as follows. The action of alkali- and earth alkali-metal ions on the luminescence is in the following order:

1. The exciting action in the initial stadium

Cations........................Mg > K, Cs > Na, Li, Ca, Sr, Ba > Rb
Anions..............................I, NO_3 > SO_4 > Br, Cl, S_2O_3 > NO_2

2. The action concerned in the persistence of the spontaneous illumination

Cations........................Mg, Li > Na, K, Ca, Sr > Cs, Ba, Rb
Anions............................SO_4, S_2O_3 > I, Br, Cl, NO_3 > NO_2

3. The action concerned in the persistence of the luminous power, i.e., irritability against a mechanical stimulus

Cations.......................Mg, Li > Na, K, Ca, Sr > Cs > Ba, Rb
Anions.............................SO_4 > S_2O_3, I, Br, Cl, NO_3 > NO_2

Thus the Mg ion is the most active.

TABLE 8

The action of anions on luminescence. Experimental temperature 14.8° to 15.8°C.

SALT	CONCENTRATION ($\Delta = -0.93$°C.) IN PER CENT	INTENSITY OF ILLUMINATION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER
			hours	*minutes*	*hours*
NaCl	1.62	+		30	1.5
NaBr	2.71	+		30	1
$NaI+2H_2O$	4.95	++		30	1
NaSCN	2.29	++		30	1.5
$NaNO_3$	2.51	++		30	1.5
$NaHCO_3$	2.60	+	1	0	1.5
$NaCH_3CO_2+3H_2O$	3.31	+		5	0.5
$NaNO_2$	1.95	−		0	0
$Na_2SO_4+10H_2O$	7.70	++	1	0	>4
$Na_2S_2O_3+5H_2O$	6.10	+	1	0	1
Na_2SO_3(sicc.)	3.30	−		0	0
$Na_2HPO_4+12H_2O$	8.35	+	1	0	2
$NaH_2PO_4+H_2O$	4.15	−		0	0
Na-tartrate	3.69	+		20	0.5
$MgSO_4+7H_2O$	11.4	++	2	0	>4
Sea water	½ dil.	+	1	30	>4

Ferrous, manganese and zinc ions, the bivalent heavy metal ions used, destroy the luminous power with some initial excitation.

c. The co-action of ions of compounds on luminescence. The co-action of ions contained in the sea water was studied. First of all it was necessary to learn the ionic composition of the sea water. So, before the experiments, the sea water taken from the environment of Watasenia was analyzed. The result is as follows:

Date of collection of the sea water: June 30, 1915. Fair weather.
Specific gravity of the sea water: 1.0255 at 15°C.
Osmotic pressure of the sea water: $\Delta = -1.867°C$.
Salts in 100 cc.:

	grams
NaCl	2.5105
KCl	0.2805
$CaSO_4$	0.1487
$MgSO_4$	0.2033
$MgCl_2$	0.2786

Then the following experiments were made in the next year.

For the sake of convenience, the following composition was assumed as that of the sea water.

	per cent
NaCl	2.51
KCl	0.28
$CaCl_2$	0.12
$MgCl_2$	0.28
$MgSO_4$	0.20
NaBr	0.05

Taking 2.51 per cent NaCl solution as the base, the other components were added in all combinations and the action of these solutions was examined.

The results of these experiments are shown in table 9. From this table it will be seen that Mg, K and Br ions act favorably, but Ca ion unfavorably upon the luminescence of the animal, when their concentrations were equal to those of the sea water.

Next, the effect of varying the ratio of some of the more important combinations of salts was studied.

1. Action of the Na + K system. After examining various ratios of NaCl and KCl concentrations, it was found that the most favorable one was 7 mol NaCl: 1 mol KCl in a rough estimation. This ratio is nearly the same as that of these two substances in the sea water (about 9 mol NaCl : 1 mol KCl). Table 10 serves as an example.

2. Action of the Na + Mg system. Various ratios of NaCl and $MgCl_2$ concentrations were examined and it was found that the higher the concentration of $MgCl_2$, the more favorable was the action of the solution. Table 11 will serve as an example.

3. Action of the Na + K + Mg system. When different amounts of $MgCl_2$ were added to $\frac{1}{2}$ mol NaCl + $\frac{1}{10}$ mol KCl solution, it was found that the favorable action of Mg ion increased in proportion to its amount. Table 12 will serve as an example.

TABLE 9

The co-action of the components of the sea water on luminescence. Experimental temperature 15.4° to 20.0°C.

SOLUTION	TIME OF PERSISTENCE OF THE ILLUMINATION		TIME OF PERSISTENCE OF THE LUMINOUS POWER
	hours	*minutes*	*hours*
$NaCl$		20	1
$NaCl+KCl$		20	3
$NaCl+CaCl_2$		10	0.5
$NaCl+MgCl_2$		10	2
$NaCl+MgSO_4$		10	2
$NaCl+NaBr$		10	2
$NaCl+KCl+CaCl_2$		5	0.5
$NaCl+KCl+MgCl_2$		10	12
$NaCl+KCl+MgSO_4$		20	10
$NaCl+KCl+NaBr$		20	5
$NaCl+CaCl_2+MgCl_2$		10	0.5
$NaCl+CaCl_2+MgSO_4$		10	0.5
$NaCl+CaCl_2+NaBr$		10	0.5
$NaCl+MgCl_2+MgSO_4$		10	2
$NaCl+MgCl_2+NaBr$		10	3
$NaCl+MgSO_4+NaBr$		10	2
$NaCl+KCl+CaCl_2+MgCl_2$		10	12
$NaCl+KCl+CaCl_2+MgSO_4$		10	10
$NaCl+KCl+CaCl_2+NaBr$		5	5
$NaCl+KCl+MgCl_2+MgSO_4$	2		14
$NaCl+KCl+MgCl_2+NaBr$	2		12
$NaCl+KCl+MgSO_4+NaBr$	2		12
$NaCl+CaCl_2+MgCl_2+MgSO_4$		10	7
$NaCl+CaCl_2+MgCl_2+NaBr$		10	4
$NaCl+CaCl_2+MgSO_4+NaBr$		10	4
$NaCl+MgCl_2+MgSO_4+NaBr$		10	8
$NaCl+KCl+CaCl_2+MgCl_2+MgSO_4$		10	10
$NaCl+KCl+CaCl_2+MgCl_2+NaBr$		10	10
$NaCl+KCl+CaCl_2+MgSO_4+NaBr$		10	10
$NaCl+KCl+MgCl_2+MgSO_4+NaBr$	8		14
$NaCl+CaCl_2+MgCl_2+MgSO_4+NaBr$		5	8
$NaCl+KCl+CaCl_2+MgCl_2+MgSO_4+NaBr$		20	10
Sea water		20	8

4. Action of the Na + Ca system. Various ratio of $NaCl$ and $CaCl_2$ concentrations were examined, and it was found that the solution which contained $NaCl$ and $CaCl_2$ in ratio of 3 : 1–31 : 1 in mol concentration was more favorable than pure NaCl or $CaCl_2$ solution. Table 13 will serve as an example.

TABLE 10

Action of the Na+K *system on luminescence. Experimental temperature 17.5° to 20.0°C.*

CONCENTRATION OF NaCl + KCL IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*hours*
NaCl+KCl	$\frac{1}{2} + 0$	10	0.5
	$\frac{31}{64} + \frac{1}{64}$	10	0.5
	$\frac{15}{32} + \frac{1}{32}$	30	6.0
	$\frac{7}{16} + \frac{1}{16}$	60	8.0
	$\frac{3}{8} + \frac{1}{8}$	60	6.0
	$\frac{1}{4} + \frac{1}{4}$	30	4.0
	$\frac{1}{8} + \frac{3}{8}$	30	0.5
	$0 + \frac{1}{2}$	30	0.5
Sea water		10	7.0

TABLE 11

Action of the Na+Mg *system. Experimental temperature 24° to 28°C.*

CONCENTRATION OF NaCl + $MgCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION		TIME OF PERSISTENCE OF LUMINOUS POWER	
		hours	*minutes*	*hours*	*minutes*
NaCl+$MgCl_2$	$\frac{1}{2} + 0$		5		20
	$\frac{63}{128} + \frac{1}{128}$		5	1	
	$\frac{31}{64} + \frac{1}{64}$		5	1	
	$\frac{15}{32} + \frac{1}{32}$	1	0	1	
	$\frac{7}{16} + \frac{1}{16}$	1	0	2	
	$\frac{3}{8} + \frac{1}{8}$	1	30	2	
	$\frac{1}{4} + \frac{1}{4}$	1	30	2	
	$0 + \frac{1}{2}$	1	0	1	
Sea water			20	2	

5. *Action of the K + Ca system.* Various ratio of KCl and $CaCl_2$ concentrations were tested and it was found that the more K ion and the less Ca ion, the more favorably the solution acted. Table 14 will serve as an example.

6. *Action of the Mg + Ca system.* It was found that the more the concentration of Ca ion, the more unfavorably the solution acted. Table 15 will serve as an example.

TABLE 12

Action of the Na+K+Mg system. Experimental temperature 24° to 28°C.

CONCENTRATION OF NaCl + KCl + MgCl$_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		hours	*hours*
NaCl+KCl+MgCl$_2$	$\frac{1}{2}+\frac{1}{10}+0$	1	2
	$\frac{1}{2}+\frac{1}{10}+\frac{1}{100}$	1	2
	$\frac{1}{2}+\frac{1}{10}+\frac{1}{50}$	1	2
	$\frac{1}{2}+\frac{1}{10}+\frac{1}{25}$	1	2
	$\frac{1}{2}+\frac{1}{10}+\frac{1}{10}$	1.5	3
	$\frac{1}{2}+\frac{1}{10}+$ [illegible]	2	3
	$\frac{1}{2}+\frac{1}{10}+$ [illegible]	2	3
Sea water		1	2

TABLE 13

Action of the Na+Ca system. Experimental temperature 22.5° to 24.4°C.

CONCENTRATION OF NaCl + CaCl$_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER	
		minutes	*hours*	*minutes*
NaCl+CaCl$_2$	$\frac{1}{2}+0$	10		30
	$\frac{49}{100}+\frac{1}{100}$	10		30
	$\frac{11}{24}+\frac{1}{24}$	10	2	0
	$\frac{9}{20}+\frac{1}{20}$	10	1	30
	$\frac{4}{10}+\frac{1}{10}$	10	1	30
	$\frac{3}{8}+\frac{1}{8}$	10	1	30
	$\frac{1}{4}+\frac{1}{4}$	20		20
	$\frac{1}{8}+\frac{3}{8}$	20		20
	$0+\frac{1}{2}$	25		25
Sea water		25	3	

7. *Action of the Na + K + Ca system.* To test the action of this system, various amounts of CaCl$_2$ were added to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{10}$ mol KCl, and the effects of these solutions were examined. The result was that an addition of CaCl$_2$, even in small amounts, made the solution unfavorable for luminescence. It is, therefore,

possible to assume that the Ca ion content of the sea water (about $\frac{1}{100}$ mol. $CaCl_2$) is unfavorable to the luminescence of Watasenia. Table 16 will serve as an example.

TABLE 14

Action of the K+Ca *system. Experimental temperature 18.2° to 19.6°C.*

CONCENTRATION OF $KCl + CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$KCl+CaCl_2$	$\frac{1}{2} + 0$	5	30
	$\frac{5}{12} + \frac{1}{12}$	5	30
	$\frac{2}{6} + \frac{1}{6}$	5	45
	$\frac{1}{4} + \frac{1}{4}$	5	30
	$\frac{1}{6} + \frac{2}{6}$	<5	10
	$\frac{1}{12} + \frac{5}{12}$	<5	10
	$0 + \frac{1}{2}$	<5	10
Sea water		5	>60

TABLE 15

Action of the Mg+Ca *system. Experimental temperature 18.2° to 19.6°C.*

CONCENTRATION OF $MgCl_2 + CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$MgCl_2+CaCl_2$	$\frac{1}{3} + 0$	10	40
	$\frac{7}{24} + \frac{1}{24}$	20	40
	$\frac{3}{12} + \frac{1}{12}$	20	30
	$\frac{5}{24} + \frac{3}{24}$	20	30
	$\frac{1}{6} + \frac{1}{6}$	20	20
	$\frac{3}{24} + \frac{5}{24}$	10	20
	$\frac{1}{12} + \frac{3}{12}$	5	20
	$\frac{1}{24} + \frac{7}{24}$	5	20
	$0 + \frac{1}{3}$	5	10
Sea water		5	>60

8. Action of the Na + Mg + Ca system. It was found that an addition of $CaCl_2$ to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{20}$ mol $MgCl_2$ also made the solution unfavorable for luminescence. Table 17 will serve as an example. The absolute value of the time in this table is shortened due to a high experimental temperature.

9. Action of the Na + K + Mg + Ca system. Lastly it was found that an addition of $CaCl_2$ to the solution of $\frac{1}{2}$ mol NaCl + $\frac{1}{20}$ mol KCl + $\frac{1}{20}$ mol $MgCl_2$ also made the solution unfavorable for the luminescence of Watasenia. Table 18 will serve as an example.

TABLE 16

Action of the Na+K+Ca *system. Experimental temperature 17.0° to 22.2°C.*

CONCENTRATION OF NaCl + KCl + $CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION		TIME OF PERSISTENCE OF LUMINOUS POWER
		hours	*minutes*	*hours*
$NaCl+KCl+CaCl_2$	$\frac{1}{2}+\frac{1}{20}+0$	1		8
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{200}$	1		4
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{100}$	1		4
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{50}$	1		4
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{40}$	1		4
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{20}$	1		4
Sea water			40	4

TABLE 17

Action of the Na+Mg+Ca *system. Experimental temperature 26 to 28°C.*

CONCENTRATION OF NaCl + $MgCl_2$ + $CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$NaCl+MgCl_2+CaCl_2$	$\frac{1}{2}+\frac{1}{20}+0$	40	40
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{200}$	5	40
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{100}$	5	40
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{50}$	5	30
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{40}$	5	20
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{25}$	5	20
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{15}$	5	20
	$\frac{1}{2}+\frac{1}{20}+\frac{1}{2}$	5	20
Sea water		5	>60

The results mentioned above may be summed up as follows: When all saline components of the sea water (NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and NaBr) are contained in a solution in the same concentration as in the sea water, the favorable ions for the production of light are in the order of Mg, K and Br, while the most unfavorable one is Ca.

The most favorable ratio of concentration of NaCl and KCl agrees with the ratio in which these two substances are found in sea water.

The favorable action of Mg ion on luminescence increases in proportion to its concentration, quite independently of the other co-existing ions.

Ca ion diminishes the favorable action of K or Mg ion on luminescence.

TABLE 18

Action of the Na+K+Mg+Ca *system. Experimental temperature 26° to 28°C.*

CONCENTRATION OF $NaCl + KCl + MgCl_2 + CaCl_2$ IN MOL.		TIME OF PERSISTENCE OF ILLUMINATION	TIME OF PERSISTENCE OF LUMINOUS POWER
		minutes	*minutes*
$NaCl+KCl+MgCl_2+CaCl_2$	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + 0$	40	60
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{384}$	5	40
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{192}$	5	40
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{96}$	5	40
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{48}$	5	40
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{24}$	5	20
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{12}$	5	20
	$\frac{1}{2} + \frac{1}{20} + \frac{1}{20} + \frac{1}{6}$	5	20
Sea water		5	>60

SUMMARY

1. Hypotonic sea water lower than $\Delta = -1.8°C.$ (the osmotic pressure of normal sea water) is unfavorable for the maintenance of life of Watasenia. An osmotic pressure lower than $\Delta = -1.5°C.$ shortens the length of its life remarkably. This is the main reason why this animal cannot live in the upper layer of the sea water, which has a very low osmotic pressure.
2. The descriptions of luminescence of the living Watasenia are to be found in the text.
3. The phenomenon of luminescence in this animal is of the intracellular type. The luminous organ of this animal, therefore, is not suitable for extraction of the luminous substances.
4. The phenomenon of luminescence of this animal is evidently due to an oxidation. The disappearance of illumination is observed in the case of absence of oxygen and its reappearance on admission of oxygen.

5. Alcohol, ether and chloroform inhibit this phenomenon in a few minutes, though in the first stage of the process they excite the production of light to a certain degree. The power of illumination is quickly recovered on the removal of the narcotics.

6. The most favorable temperature for the production of light extends from 16° to 31°C. A lower or higher temperature than the above gradually diminishes the illumination and ultimately puts an end to it. This action is reversible. A higher temperature than 49°C. destroys the luminous power owing to heat coagulation.

7. Direct sunlight has no influence upon the luminescence of Watasenia.

8. The most favorable osmotic pressure for the production of light is from 0.2 times to 1.5 times of that of the sea water ($\Delta = -1.8°C.$). Hypotonic or hypertonic pressures lower or higher than these values inhibit the production of light. Initial excitation is, however, seen in the former case but not in the latter.

9. Both hydrogen and hydroxyl ions first excite the production of light, and then inhibit it after a certain time. Hydrogen ions destroy the luminous power while hydroxyl ions have no injurious action upon it, when they are in less concentration than $\frac{1}{50}$ normal.

10. The action of alkali and earth alkali metal ions on the luminescence of the animal is in the following order:

The exciting action in the initial stadium:

Cations .... Mg > K, Cs > Na, Li, Ca, Sr, Ba > Rb
Anions........................ I, $NO_3 > SO_4 >$ Br, Cl, $S_2O_3 > NO_2$

The action concerned in the persistence of the spontaneous illumination:

Cations .... Mg, Li > Na, K, Ca, Sr > Cs, Ba, Rb
Anions........................ SO_4, $S_2O_3 >$ I, Br, Cl, $NO_3 > NO_2$

The action concerned in the persistence of the luminous power:

Cations .... Mg, Li > Na, K, Ca, Sr > Cs > Ba, Rb
Anions........................ $SO_4 > S_2O_3$, I, Br, Cl, $NO_3 > NO_2$

Among them the Mg ion is the most favorable.

Ferrous, manganese and zinc ion, the bivalent heavy metal ions tested, destroy the luminous power with some initial excitation.

11. When all saline components of the sea water, i.e., NaCl, KCl, $CaCl_2$, $MgCl_2$, $MgSO_4$ and NaBr are contained in a solution in the

same concentration as in the sea water, the favorable ions for the production of light are in the order of Mg, K and Br, while the most unfavorable one is Ca.

The most favorable ratio of concentration of NaCl and KCl agrees with the ratio in which these two substances are found in sea water.

The favorable action of the Mg ion on luminescence increases in proportion to its concentration, quite independently of the other co-existing ions.

The Ca ion diminishes the favorable action of the K or Mg ion on the luminescence of the animal.

In conclusion the writer wishes to express his thanks to Prof. H. Ishikawa for his kind direction and also to Prof. C. Ishikawa for his information about the morphology of Watasenia.

BIBLIOGRAPHY

(1) **Joubin**: Bull. de la soc. scient. et med. de l'ouest, 1896, v., 1.
(2) **Hoyle**: Bull. Museum Comp. Zoöl., Harvard College, 1904, xliii, 36.
(3) **Chun**: Die Cephalopoden I. Teil: Oegopsida. Wiss. Ergebn. d. Deut. Tiefsee-Expedition, Bd. 18.
(4) **Watase**: Dobutsugaku Zassi (Journ. Zoöl. in Japan), 1905, xvii, 119.
(5) **Berry**: A catalogue of Japanese Cephalopoda, 1912.
(6) **Ishikawa**: Zoöl. Anz., 1914, xliii, 162.
(7) **Sasaki**: Dobutsugaku Zassi, 1913, xxv, 581; Journ. Coll. Agric. Tohoku Imp. Univ., Japan, 1914, vi, part 4.
(8) **Matsuno**: Toyamaken-Suisankoshusho Hokoku (Rept. of Fisheries Inst. 1912 and 1913.
(9) **Joubin**: Nouvelle recherches sur l'appareil lumineux des Céphalopodes du genre Histioteuthis, (Dissért.), 1894.
(10) **Dubois**: C. R. Soc. Biol. 1885, xxxvii, 559. See also **Dubois**: La vie et la lumière, 1914, 127.
(11) **Harvey**: This Journal, 1917, xlii, 318, 342 and 349.
(12) **Pütter**: Zeitschr. f. allg. Physiol., 1905, v, Sammelref. 17.
(13) **Kölliker**: Verhandl. d. physik-med. Gesell. in Würzburg, 1858, viii, 217.
(14) **Mangold**: Pflüger's Arch., 1907, cxviii, 613. Many other references about the bioluminescence are to be found in **Mangold**: Die Produktion von Licht in Winterstein's Handb. d. vergleich. Physiol., 1910–14, iii.

EXPERIMENTAL MAMMALIAN POLYNEURITIS PRODUCED BY A DEFICIENT DIET

CARL VOEGTLIN AND G. C. LAKE

From the Hygienic Laboratory, U. S. Public Health Service

Received for publication October 29, 1918

INTRODUCTION

Progress in medicine is intimately connected with animal experimentation, particularly the reproduction of human diseases in the lower animals. It seems almost superfluous to call attention to the great benefit which modern medical science has derived from the successful reproduction in the common laboratory animals of such infectious diseases as rabies, tuberculosis, trypanosomiasis, syphilis, etc. Discoveries of this nature are usually followed by more rational methods of diagnosis, prevention and treatment. This applies equally well to the diseases of dietary origin, which group includes the so-called deficiency diseases of which beri beri is the best known example. Thus it cannot be questioned that Eijkman's discovery that polyneuritis in fowls could be induced by an exclusive diet of polished rice has very materially contributed to our present conception of the etiology of beri beri and its prevention.

The study of deficiency polyneuritis is also intimately connected with the more recent development of the physiological aspects of nutrition inasmuch as it has been shown that beri beri is due to a deficiency of the diet in a definite substance (antineuritic vitamine), which is essential for normal nutrition. From this standpoint the study of beri beri in animals will undoubtedly shed some light on the physiological function of the antineuritic vitamine. All we know at the present time regarding this function is that a certain minimal amount of this substance must be present in the diet in order to permit normal growth of the young and the maintenance of weight and health of the adult animal and man.

Although birds are very satisfactory for certain work on polyneuritis, their usefulness is obviously limited inasmuch as their anatomy and physiological behavior differ considerably from those of mammalia. The present investigation aimed, therefore, primarily at the production of deficiency polyneuritis in animals which were more closely related to man and which could be used to better advantage in studies on experimental polyneuritis and the physiological function of the antineuritic vitamine.

Literature referring to mammalian polyneuritis. A perusal of the literature on beri beri and deficiency polyneuritis shows that several investigators have observed symptoms and pathological lesions in mammals, resembling very closely those characteristic of beri beri. However, no systematic attempt seems to have been made to identify this condition as true polyneuritis due to a dietary deficiency.

De Lacerda ('85) reports a study of a disease extensively prevalent among horses and hogs in Brazil. This author calls attention to the great similarity of this disease with beri beri, and also emphasizes the fact that carnivorous animals seemed to be refractory to it.

Hose ('05) very briefly refers to a feeding experiment with three monkeys (*Macacus nemestrinus*) on rice. "One of the monkeys exhibited no special symptoms, but the other two developed some of the characteristic nervous symptoms of beri beri."

Braddon ('07) in his book on the cause and prevention of beri beri graphically describes a disease which he observed in India among horses fed on rice paddy. From his description, he was dealing with a disease very similar to beri beri.

Schaumann ('10) reports experiments on a few rats and dogs which he fed on "denatured" horse meat. The animals lost body weight and after several weeks exhibited paralytic symptoms. The peripheral nerves revealed myelin degeneration of a rather mild degree.

Shiga and Kusama ('11) fed two Japanese monkeys (*Macacus cynomolgus*) exclusively on boiled rice. One of the animals became emaciated and on the 37th day of the experiment developed paretic symptoms in his hind legs. The other monkey died after five months from generalized tuberculosis, without having shown any paralytic symptoms. The necropsy findings of the paretic animal included a slight oedematous condition of the lower parts of the hind legs, increased pericardial fluid, enlargement of the heart and oedema of the posterior lobes of the lungs. Histological examination of the peripheral nerves showed a number of degenerated fibers.

Rommel and Vedder ('15) report a few experiments in which beri beri-like symptoms were observed in pigs fed on rice.

Andrews ('12) produced polyneuritis in seven young puppies which were nursed by Philippine mothers whose infants had died of beri beri.

Osborne and Mendel and McCollum and his collaborators in their work on growth state that they have frequently observed polyneuritis in albino rats which were fed on a deficient diet. We are able to confirm these findings, but we also should like to point out the limited usefulness of rats on account of their small size.

In summing up this review of the literature pertaining to experimental mammalian polyneuritis it is evident that, so far, no method is available by which this disease can be produced with any degree of certainty in the larger mammalia nor have most of the previous investigators clearly demonstrated that the disease which they observed was due solely to a deficiency of the diet in antineuritic vitamine.

EXPERIMENTAL PART

The experiments which will be reported in the following pages were carried out on dogs, cats and albino rats. From preliminary experiments it soon became evident that cats seemed to respond to a deficient diet with the greatest regularity and for this reason most of the work dealt with this species.

In regard to the selection of the food, the dietary habits of the various animals had to be taken into consideration. It stands to reason that a carnivorous animal cannot be fed satisfactorily on vegetable foods to which it is not accustomed. It should therefore be emphasized that the successful prosecution of work of this kind depends mainly on a proper selection of the diet. For instance, adult dogs and cats can be kept for many weeks and months in perfect health on an exclusive diet of beef. This food evidently fulfills all the dietary requirements for this species and was, therefore, adopted in this investigation. Rats, being omnivorous animals, can be maintained for a limited period on meat, but as already pointed out by Watson and Hunter ('06), prolonged meat feeding ultimately leads to various pathological changes.

The question arose as to whether it was possible to destroy the antineuritic substance of beef without otherwise altering the dietary value of this food. As will be seen from the results to be described, meat, even when heated for three hours at 120°C., is still a fairly satisfactory food for cats although its antineuritic power is somewhat reduced by the heating. As exposure of the beef to a relatively high temperature did not seem to yield a product devoid of antineuritic power, it was thought that a combination of heat and alkali might accomplish this purpose. Voegtlin, Sullivan and Myers ('16), for instance, had discovered the fact that the antineuritic substance of "whole" cornmeal is destroyed or inactivated in baking cornbread in the presence of alkali (sodium carbonate). Various other investigators, working on the chemical isolation of the antineuritic vitamine, had also recognized the deleterious action of alkalies on vitamine solutions.

On the basis of these considerations and Schaumann's experiments, the meat was prepared in the following manner: Lean beef[1] is put through a hashing machine. It is then treated under stirring with a 10 per cent solution of sodium carbonate until the mixture is distinctly alkaline to litmus paper. The meat is then heated in an autoclave for three hours at 120°C. After cooling, sufficient dilute hydrochloric acid is added to neutralize the mixture, using litmus paper as an indicator.

In order to determine whether heat alone, in the absence of an alkaline reaction, might destroy the physiological activity of the antineuritic vitamine, a number of animals were fed on beef which had been heated for three hours at 120°C without the previous addition of sodium carbonate.

Moreover, the possibility of a combined action of heat and alkali on meat constituents other than the antineuritic vitamine had to be taken into consideration. It was possible, although not very probable, that the symptoms observed in animals fed with alkali-treated meat might have been due to a destruction of certain essential amino acids or the fat-soluble vitamine. The first possibility was met by adding purified casein to the alkali sterilized meat; the second by adding a certain percentage of butter, a food which is known to contain a considerable amount of the fat-soluble vitamine.

The cats, dogs and rats were kept during the whole period of the experiment in small, well ventilated rooms. During the colder season of the year it was found necessary to provide sufficient heat to prevent the occurrence of distemper. The cats and dogs, fed on the deficient diet, seemed extremely susceptible to this disease and we lost a great number of the animals from this cause until the rooms were properly heated. After this precaution was taken there were no further cases of distemper. A sufficient supply of fresh water was provided for all animals.

The following tables, protocols and charts illustrate the main results.

---

[1] It is important that most of the fat be removed from the meat as otherwise the alkali will cause considerable saponification during the subsequent heating. When this precaution is neglected the animals do not seem to relish the meat and often refuse to eat it altogether.

TABLE 1

Polyneuritis produced in cats by a diet of lean beef, heated for three hours at 120°C. in the presence of sodium carbonate

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
2	30	45	*30th day:* Complete paralysis of legs with intermittent convulsive attacks *31st day:* Died *Necropsy:* No marked, gross pathological findings
5	22	33	*22nd day:* Slight paralysis of legs and convulsive seizure *23rd day:* Received 20 cc. autolyzed yeast filtrate by stomach tube *24th day:* Considerably improved. Has distemper *25th day:* Died. Slight gastro-enteritis Vagus and sciatic: Myelin degeneration
3	27	18	*27th day:* Complete leg paralysis. On being handled, develops clonic spasm. Pupils react very sluggishly to strong light. Given 20 cc. autolyzed yeast filtrate by stomach tube *28th day:* Considerably improved. Sits in normal position. No convulsions. Has distemper *29th day:* No convulsions or marked paralysis, but appears sick *30th day:* Found dead Purulent tracheo-bronchitis. Lungs: passive congestion and oedema. Sciatic: mild myelin degeneration
6	21	38	*21st day:* Marked leg paralysis and clonic convulsions *22nd day:* Signs of distemper noticed. Paralytic symptoms more pronounced *23rd day:* Receives 20 cc. autolyzed yeast filtrate by stomach tube. Most of this is lost by vomiting *24th day:* Slightly improved. 20 cc. yeast filtrate *25th day:* Paralysis much improved. Distemper worse *26th day:* Died. Purulent bronchitis. Pulmonary congestion. Slight gastritis. Sciatic and vagus show some degenerated fiber

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
7	18	27	*18th day:* Spasticity of hind legs, slight clonic convulsions *20th day:* Condition unchanged. Resp. 28; pulse 198; rect. temp. 38.5°C. *21st day:* Paralysis worse; resp. 33; pulse 186; rect. temp. 38°C. *22nd day:* Severe convulsions; resp. rapid and labored *23rd day:* Found dead. Sciatic: foamy appearance, mild degeneration
8	18	17	*18th day:* Walks with marked incoördination and weakness of hind legs; falls often; resp. 32; pulse 172, rect. temp. 36.5°C. Knee jerks present *20th day:* General appearance about same as last noted. Still shows incoördination. Has convulsions lasting a few seconds *21st day:* Much worse, resp. 70, pulse 68; rect. temp. 34°C. *22nd day:* Found dead. No marked gross pathological findings. Sciatic slightly degenerated
9	14	23	*14th day:* Semi-comatose; unable to walk; knee reflex present. Slight convulsions. Resp. 18; pulse 48, rect. temp. below 32°C. Died at 10 p.m. No marked gross pathological findings except considerable emaciation and slight nephritis. Sciatic: foamy appearance of many fibers
10	24	30	*24th day:* Walks with marked spasticity of legs *25th day:* Found dead *Necropsy:* Purulent bronchitis; otherwise normal. Sciatic shows many fibers with segmentation and foamy-like structure
11	19	32	*19th day:* Unsteady gait, especially marked in hind legs *20th day:* Definitely worse, plaintive cry, cannot take more than a few steps without falling, refuses food *21st day:* Unable to walk, resp. 45, somewhat labored. Given 20 cc. autolyzed yeast filtrate by stomach tube at 11.35 *22nd day:* Much improved, walks about without much difficulty, appetite returned. Seems to relish sterilized meat

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
11	19	32	*24th day:* Walks about normally, seems to be in fair condition. Given 15 cc. autolyzed yeast filtrate. *25th day:* Not a trace of paresis, seems quite lively. Given 15 cc. autolyzed yeast filtrate *26th day:* Normal appearance, 15 cc. autolyzed yeast filtrate. Eats normally *27th day:* Seems perfectly normal *40th day:* Still in excellent condition. Is fed from now on daily with alkali sterilized meat to which 3 cc. of autolyzed yeast filtrate per kilo of body weight is added. Has gained considerably in body weight *140th day.* Still in excellent condition. Body weight 18 per cent above initial weight. Administration of yeast filtrate is discontinued *211th day:* Refuses food. Seems to be weak. Has no convulsions *215th day:* Staggering gait *216th day:* Found dead. Loss of weight 23 per cent
12	23	8	*23rd day:* Marked incoördination when attempting to walk, falls over as if intoxicated. Clonic convulsions, opisthotonus at times. Refused to eat for last two days. Given 20 cc. autolyzed yeast filtrate. Complete recovery of general health and body weight during the following weeks on a diet of alkali sterilized meat + 1.5 cc. yeast filtrate per kg. body weight *140th day:* Animal in excellent condition
13	20	13	*20th day:* Marked ataxia, from time to time has severe clonic convulsions, during which head and neck are bent forward. Plaintive cries. Resp. 25, pulse 180; rect. temp. 37.3°C. Refuses food. Legs show spasticity. Given 20 cc. yeast vitamine ($FeCl_3$, vitamine no. 3) *21st day:* Can walk, shows but little incoördination. Spasticity of legs has almost completely disappeared. No more convulsions. Given 20 cc. yeast vitamine ($FeCl_3$ vitamine no. 3.)

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
13	20	13	*22nd day:* Improved, but does not eat; given 20 cc. autolyzed yeast filtrate. Resp. 21; pulse 162; rect. temp. 38.2°C. *23rd day:* Condition slightly worse; one convulsion; given 25 cc. autolyzed yeast filtrate *24thd day:* Seems very much improved; ate some food; does not cry; pulse 134; rect. temp. 38.8°C. Accidentally killed by passing stomach tube into trachea during administration of yeast filtrate
14	24	25	*24th day:* Lost appetite during last week. Shows very definite spastic condition of legs. Arches back and shows typical high stepping gait in hind legs. Pulse 192; rect. temp. 38.6°C. *25th day:* Condition unchanged *26th day:* Symptoms more pronounced. Considerable spasticity; convulsions; given 25 cc. autolyzed yeast filtrate, most of which was vomited up a few minutes later. An additional 15 cc. was given and retained *27th day:* Appears much improved; walks about normally; does not show much spasticity; from this date on animal made rapid recovery, so far as general condition and body weight are concerned, without any change in diet except that cat receives daily 4 cc. autolyzed yeast filtrate per kilo body weight *121st day:* Animal in excellent condition. Gain of body weight 25 per cent
15	18	15	*18th day:* Considerable ataxia when walking. Few severe convulsions lasting a few seconds each are noted. Plaintive cry. Pulse 200; regular; resp. 36; rect. temp. 37.3°C. Paralytic symptoms slightly improved, but after some exercise symptoms gradually grow worse. Marked flow of thick saliva *19th day:* Resp. shallow and irregular; faulty pulse; rect. temp. 38.5°C.; given 15 cc. yeast vitamine ($FeCl_3$ vitamine no. 3, equivalent to 37.5. autolyzed yeast filtrate)

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS days	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
15	18	15	*20th day:* Walks about room with considerable incoordination but is very much improved. Still refuses food. This animal gradually improved after receiving another dose of ferric chloride vitamine, followed by daily administration of 4 cc. autolyzed yeast filtrate per kg. body weight The body weight returned normal and the symptoms entirely disappeared *110th day:* Animal in excellent condition. Escapes
46	53	16	*53rd day:* Hind legs completely paralyzed. Unable to stand. Occasional clonic convulsions lasting for a few seconds. Pupils dilated; react very sluggishly to strong light *54th day:* Found dead *Necropsy finding:* lungs slightly congested. Increase in pericardial fluid. Fatty liver. Kidney pale. Gall bladder distended. Gastro-intestinal tract normal. Sciatic shows extensive myelin degeneration. Vagus only slightly degenerated
47	40	11	*40th day:* Several severe convulsions lasting few seconds. Walks with practically normal gait during intervals. Pulse rate high (cannot be counted) and resp. normal *41st day:* When attempting to walk falls over. Paralysis especially noticeable in hind legs. Fairly well nourished. Pupils enlarged; react very slowly and incompletely to strong sunlight. Pulse 240, regular; resp. 52; shallow, irregular, with tendency to stop during expiration. Plaintive cry. Convulsions can be elicited by handling the animal. Excessive secretion of thick saliva. Used for blood pressure experiment *Necropsy findings:* Increase in clear pericardial fluid. Slight pulmonary congestion. Fatty liver Spleen, stomach and intestine normal. Kidney pale. Sciatic shows diffuse degeneration. Vagus very slightly degenerated

TABLE 1—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
48	47	29	*47th day:* Animal unable to stand up; paralysis especially marked in hind legs. Pupils enlarged. Pulse very fast. Resp. 45, somewhat labored *48th day:* Condition unchanged. Paralysis more marked. No convulsions *49th day:* Found dead *Necropsy findings:* Lungs normal; heart enlarged; liver fatty; otherwise no significant changes. Sciatic: most fibers show moderate degree of myelin degeneration
51	39	24	*39th day:* Animal, after walking about room for some time, develops slight paralytic symptoms *40th day:* Convulsions have appeared. During intervals between convulsions cat stands with legs spread apart. Hind legs especially weak. When walking, hind legs give way and cat falls on floor. Pupils dilated; resp. 56; irregular, largely abdominal in character. Pulse 240 when lying quietly *41st day:* Complete paralysis of legs. Unable to stand up. Lying on side. Resp. 40, Pulse 240 *42nd day:* Found dead *Necropsy:* Fatty appearance of liver. Other organs practically normal. Sciatic shows foamy appearance and only slight degeneration
52	38	27	*38th day:* Able to stand up but falls down when attempting to walk; paralysis especially pronounced in hind legs. Arched back. Has convulsions at frequent intervals. Resp. 52, regular, largely abdominal; pulse 152, fairly regular *39th day:* Paralysis of hind legs complete. Has tendency to crawl in circle to right. Holds head to right side. Plaintive cry. Palpebral fissure enlarged and pupils dilated. Resp. 72; pulse 232. Given subcutaneously 4 cc. (80 mgm.) yeast vitamine fraction *40th day:* Seems to be slightly improved. Given 30 cc. autolyzed yeast filtrate

TABLE 1—*Concluded*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
52	38	27	*41st day:* Very marked improvement. Animal stands up and walks with pronounced ataxia. No convulsions. Given 20 cc. autolysed yeast filtrate *42nd day:* Paralytic symptoms further improved. Appetite has returned; 10 cc. autolysed yeast filtrate given. Treatment from here on omitted and cat finally died 9 days later, after return of symptoms
53	31	32	*31st day:* Swaying gait. Has convulsions from time to time. Does not appear to be in pain. Pulse 84, irregular. Respiration labored at times *32nd day:* Condition unchanged; animal used for blood pressure experiment *Necropsy findings:* Heart normal, very small congested area in right lung. Liver has fatty appearance. Spleen, intestine and kidneys normal in appearance. Sciatic shows advanced myelin degeneration. Vagus, fairly well marked degeneration with foamy appearance
54	34	21	*34th day:* Has severe leg paralysis. Unable to stand. Crawls about. Resp. is periodically very rapid and labored. Pulse 200. Animal used for blood pressure experiment. Sciatic shows very extensive myelin degeneration
55	35	10	*35th day:* Has several convulsions lasting for a few seconds each. Pulse very rapid; impossible to count; resp. normal *36th day:* Condition unchanged *37th day:* Legs show very marked paralysis; resp. irregular and labored. Heart rate very rapid. Pupils enlarged *38th day:* Symptoms still further aggravated. Animal still able to walk. Pulse 250. Resp. irregular, spasmodic at times. Has refused food for several days. Animal used for blood pressure experiment. Sciatic and vagus show moderate myelin degeneration

TABLE 2

Polyneuritis produced in cats by a diet of lean beef, heated for three hours at 120°C. in the presence of sodium carbonate. (Effect of addition of purified casein, or butterfat, or both)

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
100	56	20	(*10 per cent purified casein was added to the meat, after removal from the autoclave*) *56th day:* Considerable weakness in hind legs *57th day:* Weakness in hind legs more marked. Falls over when attempting to walk *60th day:* Symptoms have gradually increased in severity during last 3 days. Marked paralysis of the hind legs. Intermittent convulsions observed. Cat found lying on side in an unconscious condition. Pupils contracted. Corneal reflex practically absent. Respiration abdominal; shallow and irregular. Heart beat 96, regular but very feeble. Pulse in femoral artery cannot be made out. Body cold. Dies *Necropsy:* Emaciated body. Mechanical stimulation of phrenic causes contraction of diaphragm; that of sciatic, contraction of leg muscles. Lungs show small oedematous areas. Heart dilated. Increase in pericardial fluid. Liver congested, fatty appearance. Gall bladder distended. Spleen and kidney congested. Abdominal lymph glands enlarged. Stomach normal except congestion of fundus. Small intestine normal. Large intestine filled with slightly bloody faecal matter. No oedema of subcutaneous tissue. Sciatic, foamy appearance
102	31	19	(*10 per cent purified casein was added to the meat after removal from autoclave*) *31st day:* Walks with arched back, stiffness of hind legs and marked ataxia. Does not appear to be in pain *33rd day:* Has clonic convulsions, with opisthotonus. Rigidity of legs. When walking, ataxia is more marked. Falls over at times on account of hind legs being considerably paralyzed. Rect. temp. 37.5°C. Given 25 cc. melted butter by stomach tube

TABLE 2—*Continued*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
102	31	19	*34th day:* Paralysis more severe. On attempting to stand, falls on side; still has convulsions. Swaying of anterior part of body when attempting to walk. Pulse 138. Resp. normal. Pupils react very sluggishly to light. Tries to catch mouse but refuses to eat alkali treated beef *35th day:* Completely paralyzed *36th day:* Dead *Necropsy:* No gross pathological findings except that liver presents characteristic "nutmeg" appearance. Sciatic shows mild myelin degeneration
101	33	28	(*10 per cent of casein and 5 per cent of butter fat were added to the meat, after removal from the autoclave*) *33rd day:* Animal has clonic convulsions with opisthotonus and rigidity of legs. Convulsions appear suddenly during which consciousness is not lost. Immediately after the convulsions, which last for a few seconds, the cat gets up and walks about room, showing marked rigidity of legs, arched back, swaying of posterior part of body and high stepping gait. Pupils are enlarged and palpebral fissure is abnormally great (exophthalmus). Cries, but does not seem to be in pain. Tries to catch mouse, but refuses to eat sterilized meat. Sensation to pain in legs not abolished. Receives at noon by stomach tube 18 cc. of a purified yeast vitamine fraction 2 cc. of which had caused the complete recovery of a severely paralyzed pigeon within a few hours. 2 p.m. eats 150 gm. of meat mixture. *34th day:* No convulsions. Walks about room without showing any paralytic symptoms *38th day:* Animal has been free from symptoms until today. Now shows high stepping gait and slight swaying of posterior part of body. No convulsions *44th day:* Has a convulsion immediately after being taken out of cage. Recovers rapidly and attempts to walk, but falls over on left side. Left hind leg seems to be completely paralyzed. When lying quietly has normal appearance, purrs. Respiration 60 abdominal, irregular. 4.00 p.m Given per os 12 cc. yeast vitamine 123

TABLE 2—*Concluded*

LABORATORY NUMBER OF ANIMAL	FIRST APPEARANCE OF PARALYTIC SYMPTOMS	LOSS IN BODY WEIGHT IN PER CENT ORIGINAL WEIGHT	NOTES
	days		
101	33	28	*45th day:* No convulsions. Walks without showing paralysis. Appears well *54th day:* Shows slight weakness in hind legs. No convulsions. Seems fairly well *72nd day:* Seems weak, but shows also definite ataxia. Plaintive cry *73rd day:* Dead *Necropsy:* Fairly well-nourished body. Nothing abnormal except that ileum is injected throughout. Large intestine contains dry fecal matter. Considerable peritoneal fat. Sciatic, marked myelin degeneration
104	29	22	(*10 per cent casein is added to meat after its removal from the autoclave*) *29th day:* Considerable paralysis of legs. Opisthotonus. Unable to walk. Plaintive cry *32nd day:* Paralytic symptoms have gradually become more aggravated; animal found dead. *Necropsy:* Body in fair state of nutrition. No subcutaneous oedema. Fair amount of subcutaneous fat. Liver shows some fatty change. Kidney, cloudy swelling. No other changes. Sciatic, slight myelin degeneration
105 (see chart 7)	65	9	(*10 per cent casein and 5 per cent butter fat are added to meat after its removal from the autoclave*) *65th day:* Cat has been apparently well until today. 9 a.m. Slight weakness of hind legs. Droopy. 2.30 p.m. Convulsion with opisthotonus. Walks with marked incoördination *66th day:* 9 a.m. Unable to stand or walk. Hind legs completely paralyzed. Uses front legs in attempt to get up. Barely able to crawl. Still has convulsions. Refuses food *68th day:* Condition unchanged *70th day:* Stuporous. Cries as if in pain. Leg paralysis complete *71st day:* Dead *Necropsy:* Heart dilated, flabby. Liver, fatty appearance. Spleen and kidney congested. Spinal cord normal. Cerebrum congested

PROTOCOLS

Dog 3 (see chart 9)

On the 40th day a careful examination of the animal revealed no scorbutic nor neuritic symptoms.

46th day. No definite symptoms except complete loss of appetite.

47th day. Evidence of emaciation. Pulse 144. Rectal temperature 38.5°C. Very excitable. Impossible to determine rate of respiration.

49th day at 10 a.m. Lively and seems to be in good condition. Trembles. Excitable. Temperature 38.1°C.

50th day. Still refuses food but does not appear sick. Temperature 38.8°C. Pulse rate 108.

51st day. Lively, no change. Temperature 38.6°. Pulse rate 124, irregular.

52nd day. Eats well. Temperature 39°. Pulse rate 90, irregular. Respiration 20.

68th day. Has been eating well. Is lively and of normal appearance.

70th day. Appears very gaunt. Shivers. Unable to hold up his head. Gait spastic. On taking a few steps the hind legs stiffen and are dragged. Cannot stand up more than 2 minutes. Temperature 37.6°C. Pulse 96. Used for blood pressure experiment.

Dog 5 (see chart 9)

The first symptoms were observed on the 46th day of the experiment, when the dog began to refuse his food.

48th day. Pulse 120. Respiration 16. Rectal temperature 38.9°C.

50th day. Dog lively and somewhat excited. Pulse 168, slightly irregular. Rectal temperature 38.9°C. After the dog becomes more quiet pulse drops to 140.

51st day. Pulse 105. Temperature 39°C. Still refuses food but seems quite lively.

52nd day. Eats a small amount of food. Seems to be in good condition. Pulse 140. Respiration 20. Temperature 38.3°C.

53rd day. Appetite and general appearance good. Pulse 114. Respiration 15. Temperature 39°C.

54th day. Pulse 104. Irregular. Respiration 16. Temperature 38.7°C. From this time to the 70th day, dog was apparently in good condition.

70th day, 9.30 a.m. Definite symptoms consisting of marked spasticity of hind legs, increased patellar reflexes. Pulse 68, very irregular. Respiration 12. Temperature 38°C. On very slight exertion the pulse increases to 150 and the respiration to 18.

10.30 a.m. A mild convulsion. Dog used for kymograph experiment.

Dog 6. Male. 10.9 kilos (see chart 9)

Up to 45th day dog was in generally good condition. On this day at 9.00 a.m the dog was found lying on floor. Able to stand up. Walks a few steps with staggering gait and falls down again. Appears gaunt. Conjunctivitis. The gums are slightly congested but show no hemorrhages. Knee jerks are exaggerated. Refuses food.

12.30 p.m. Unable to stand. Given 50 cc. autolyzed yeast filtrate per stomach tube. Some of this yeast product was lost by vomiting (probably not more than 20 cc.)

4.30 p.m. Condition unchanged.

46th day 10.30 a.m. Appears greatly improved. Walks almost normally, but becomes easily fatigued and falls on floor. Drinks water eagerly. Pulse 122, irregular. Respiration 10. Rectal temperature 39°C.

11.30 a.m. Runs about normally.

11.50 a.m. After exercise, pulse 140; respiration 12.

1.05 p.m. Dog has been quiet for some time. Pulse 112. Respiration 8. Temperature 38.8°C.

48th day, 9 a.m. Walks almost normally. After walking about, pulse 150, less irregular than previously. Respiration 11. Temperature 38.5°C. Still refuses food.

49th day. Gait is practically normal. Pulse 175. Respiration 11. Temperature 38.7°C.

50th day. No marked change. Urine: considerable albumen, hyaline and granular casts, bile pigments, a few pus and blood cells. Fehling, negative..

51st day. Still refuses sterilized meat. No change in condition. Given 400 cc. milk + 30 cc. autolyzed yeast filtrate. Dog drinks this mixture with eagerness.

52nd day. General condition good. Has eaten some sterilized meat. Given 400 cc. milk + 30 cc. yeast filtrate. The respiration has increased to 15; the pulse is 152. Temperature 38.8°C.

53rd day. Given 400 cc. milk + 30 cc. yeast filtrate, which is taken at once. The conjunctivitis which has been treated with boric acid has practically cleared up.

54th day. Eats 475 cc. milk + 30 cc. yeast filtrate.

55th day. 475 cc. milk.

76th day. Diet changed to 400 gm. sterilized meat + orange juice. This food is eaten very well by dog.

102nd day. Dog has been in fairly good condition until a few days ago. No paralytic symptoms were observed and appetite was excellent. However, the dog developed a skin disease (mange) and gradually lost body weight. Today the animalwas found dead. Necropsy reveals nothing abnormal except a slightly inflamed condition of gastro-intestinal tract. Sciatic nerve: myelin degeneration.

Dog 7 (see chart 10)

This animal during the first part of the experiment ate the alkali-treated meat with great avidity.

On the 31st day the animal refused its food and appeared to be not as well as usual, but no paralytic symptoms were observed.

32nd day. Appears sick. Refuses food. Slight tremor of body. Falls down on running or turning quickly. Knee reflex seems to be exaggerated. Some stiffness of muscles of neck and hindlegs. Gums normal. Examination of urine shows strongly acid reaction; considerable heat-coagulable protein; bile pigment; a few granular casts. Rectal temperature 37.8°C. Respiration 20. Pulse 126, very irregular.

34th day. Condition slightly worse. Gait more unsteady. Falls over easily. Pulse 80, irregular. Respiration 20. Rectal temperature 38.3°C. Vomits.

35th day. Condition of dog becoming progressively worse. Can still walk but falls easily. Reflexes exaggerated. Pulse 130, very irregular. Respiration 20. Rectal temperature 37.6°C. Used for blood pressure experiment.

Dog 10 (see chart 10)

This animal ate well and was in good condition during the first 28 days of the experiment. From this time on the appetite decreased and on the 42nd day the dog showed, for the first time, definite signs of paresis in hind legs. On the 45th day the dog was found dead.

Necropsy: Emaciated body. No scorbutic changes. Lungs normal; heart dilated; liver, spleen and kidneys normal; stomach shows gastritis, which is largely confined to fundus region. Duodenum shows mild inflammation. Sciatic shows slight myelin degeneration. The urine of this animal was examined the day before death occurred, with the following results: specific gravity 1018; coagulable protein, trace; a few casts; Fehling, negative; acetone and diacetic, negative.

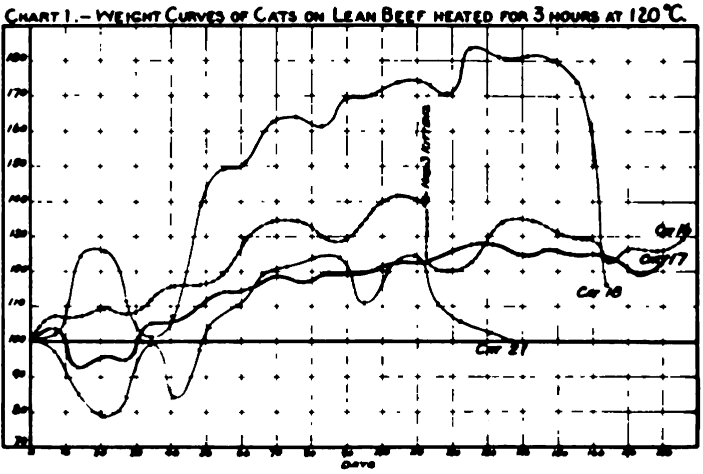


Chart 1. Illustrates the fact that adult cats can be kept for a considerable period in perfect condition, when fed exclusively on lean beef heated for 3 hours at 120°C. in the absence of free alkali). Cats 16 and 17 lived on this diet for over 180 days and were in every respect normal at the end of this period. Cat 18 died suddenly on the 164th day of the experiment, without having shown any paralytic symptoms. The cause of death in this animal could not be established but polyneuritis was not responsible for the death, as the peripheral nerves did not exhibit any myelin degeneration. Cat 16 on the 114th day of the experiment gave birth to 3 kittens which died 2 days later. The sciatic nerves of these kittens did not show any myelin degeneration. Cat 21 died on the 139th day of distemper. These experiments show that the autoclaving of the meat, *in the absence of an alkaline reaction*, does not completely destroy the antineuritic vitamine contained in fresh beef.

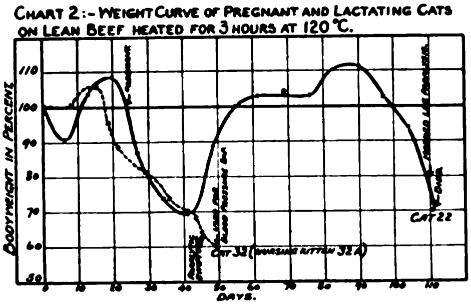


Chart 2. Shows that pregnant and lactating animals are apt to develop polyneuritis on a diet of beef which was heated for 3 hours at 120°C. Pregnancy and lactation, therefore, render the animals more susceptible to polyneuritis, an observation which is in complete agreement with the observations made on beri beri in the human. Here also it was found that pregnancy and lactation favor the appearance of the disease.

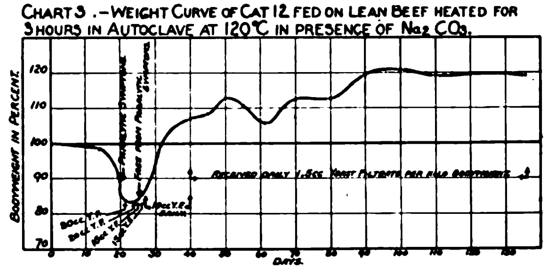


Chart 3. Illustrates the loss of weight preceding the appearance of polyneuritis on a diet of alkali-treated meat. The oral administration of autolyzed yeast relieved the symptoms, and the continued daily administration of a small amount (1.5 cc.) of autolyzed yeast fitrate prevented the reappearance of the symptoms. The animal not only recovered its original body weight but surpassed it by 20 per cent. The cat was in perfect condition on the 137th day when the experiment was discontinued.

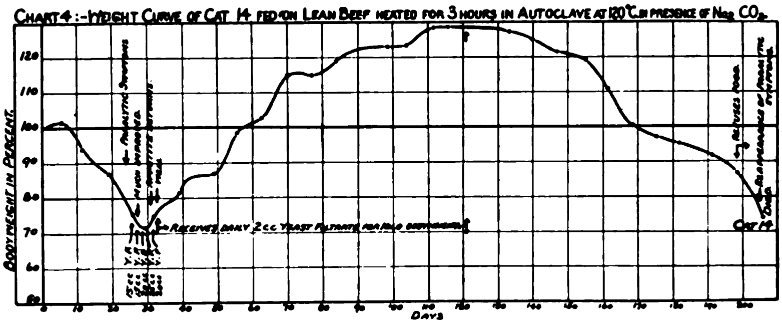


Chart 4. Shows the results of an experiment similar to that illustrated by Chart 3. It also shows that discontinuation of the autolyzed yeast filtrate is followed by a recurrence of polyneuritis.

Weight curve of cat 102 on lean beef heated for 3 hours at 120°C. in presence of Na_2CO_3. After cooling 10 per cent purified casein is added to meat.

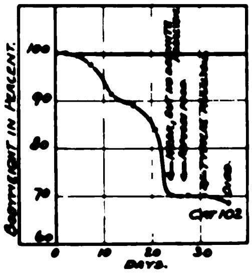


Chart 5. Illustrates the fact that the disease produced by exclusive feeding on alkali-treated beef is not due to a deficiency of the diet in certain essential amino acids, as the addition of 10 per cent purified casein to this meat does not prevent the appearance of paralytic symptoms.

Weight curve and food consumption of cat 101, fed on lean beef heated for 3 hours at 120°C. in presence of Na_2CO_3; 10 per cent purified casein added after heating.

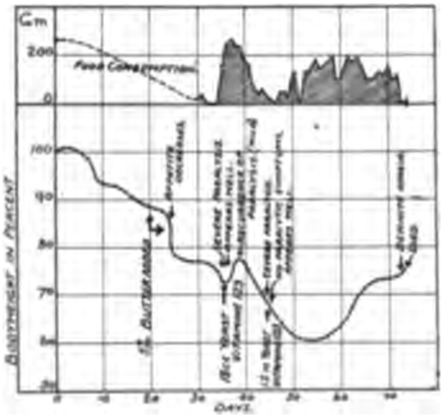


Chart 6. Shows that the addition of casein and butter fat to alkali-treated beef does not prevent the appearance of the symptoms. This is in marked contrast to the striking effect of a single comparatively small dose of an antineuritic preparation obtained from yeast; 18 cc. (equivalent to 360 mgm.) of this preparation promptly caused the complete disappearance of severe paralytic symptoms, the animal appearing perfectly normal 20 hours after the treatment. A few days later the symptoms reappeared, and on the 10th day following the first treatment the animal was again severely paralyzed; 12 cc. (equivalent to 240 mgm.) again relieved the symptoms for several days. Note influence of treatment on food consumption.

Weight curve and food consumption of cat 105 fed on lean beef heated for 3 hours at 120°C. in the presence of Na_2CO_3. After cooling 10 per cent casein and 5 per cent butter are added.

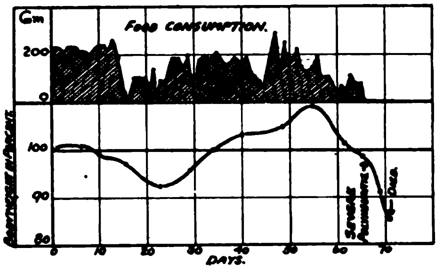


Chart 7. Shows that the addition of butter and purified casein to the alkali-treated meat does not prevent the appearance of polyneuritic symptoms. It happens that in this animal the incubation period is considerably longer than in cats fed on meat without the addition of butter and casein.

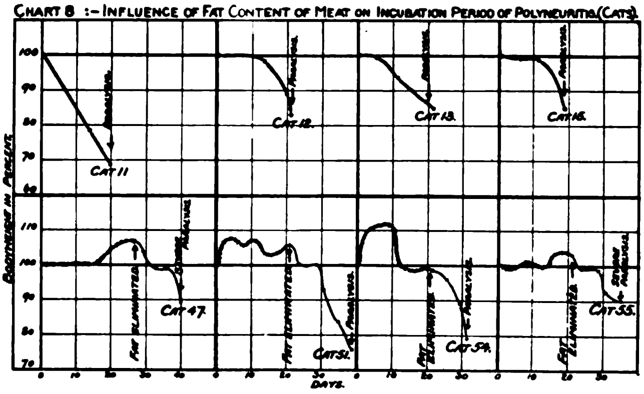


Chart 8. The upper series of animals (no. 11 to 15) was fed on beef which had been freed from fat as much as possible previous to the heating for 3 hours at 120°C. in the presence of Na_2CO_3. The second series (no. 47, 51, 54 and 55) received during a preliminary period (indicated by broken line) beef which was treated in exactly the same manner as mentioned above, with the exception that the fat had not been removed. Note the fact that on this diet the animals do not lose weight. As soon as the fat was eliminated the animals also began to lose weight and to develop polyneuritic symptoms. These results justify the conclusion that the presence of a considerable amount of fat renders the anti-neuritic vitamine of meat more resistant to the combined action of a high temperature (120°C.) and alkali.

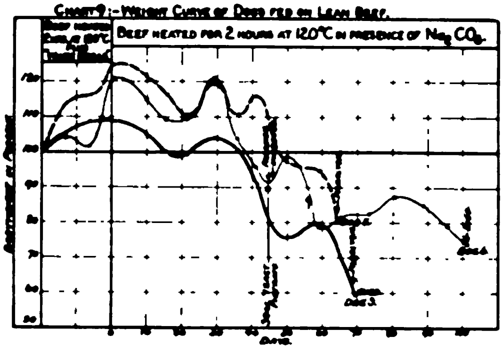


Chart 9. Illustrates the loss of body weight of dogs fed on alkali-treated meat. During a preliminary period of 20 days the animals were fed on a mixture of beef, which had been heated for 2 hours *without the previous addition of alkali*, and "white" bread. This food mixture caused an increase in body weight. On changing the diet to beef which had been heated with alkali, the animals gradually lost weight and developed typical polyneuritis. Dog 6 was severely paralyzed on the 45th day and received about 30 cc. autolyzed yeast filtrate by stomach tube. The following day the symptoms had almost completely disappeared. For further details see protocols.

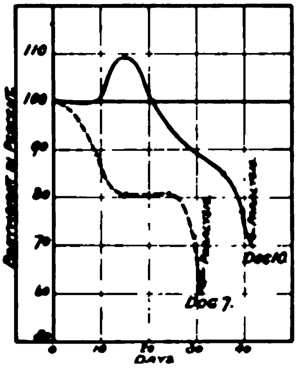


Chart 10. Weight curve of dogs fed on lean beef heated for 2 hours at 120°C. in the presence of Na_2CO_3.

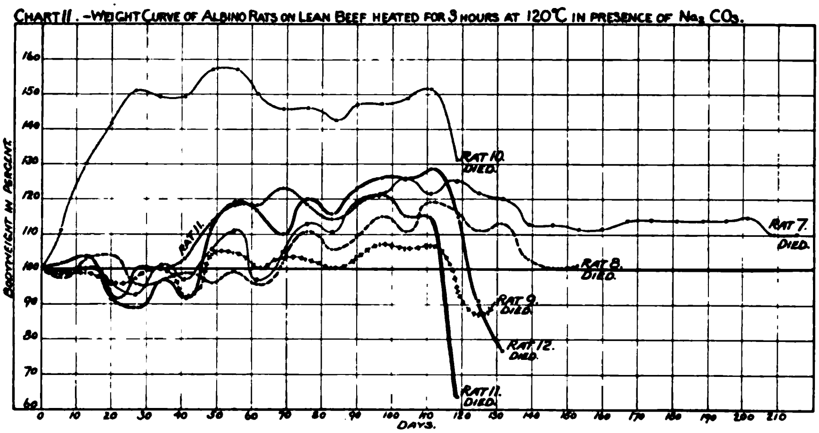


Chart 11. Shows that albino rats are much more resistant to polyneuritis than either cats or dogs. The meat fed these rats was prepared in exactly the same manner as the meat which was used for the feeding of the dogs and cats. Note the increase in body weight up to the 110th day. Rat 10 was a young animal weighing 110 gm. a tthe beginning of the experiment. This rat showed very considerable growth during the first 25 days. No symptoms characteristic of polyneuritis were observed in these animals. About the 110th day the weather became very warm (July) and the animals developed diarrhoea, which very probably was responsible for the sudden death of four of the animals.

DISCUSSION OF RESULTS

One of the most interesting points brought out by this research is the great difference in susceptibility to polyneuritis between the various species of animals. According to Fraser and Stanton ('09) the shortest incubation period for beri beri in man on a diet of highly milled rice is at least eighty-seven days. Pigeons fed on the same rice develop the disease in an average of twenty-one days, chickens in twenty-eight days. In our cats, fed on alkali-treated meat, the earliest polyneuritic symptoms were noticed on the eighteenth day. The dogs usually required about a month to six weeks for the appearance of the symptoms; and the rats (adult) lived for at least one hundred and ten days on the same deficient diet (alkali-treated beef) without showing the faintest indication of polyneuritis. One rat even lived for two hundred days, apparently in perfect health. The cats responded to the deficient diet with the greatest regularity, all of them (28) developing the disease. For unknown reasons, two of the dogs were refractory, although typical polyneuritis was observed in other dogs.

Symptomatology. As a rule the animals (dogs and cats) appeared perfectly normal and devoured the alkali-treated meat for the first week or two. Sooner or later, however, their appetite became capricious. A very marked and persistent constipation set in and the animals lost their liveliness and appeared drowsy. They then began to lose weight.

There was a great individual difference in the manner in which the paralytic symptoms made their appearance. An animal would seem to be normal until it was taken out of the cage, when it would suddenly develop a tonic convulsion which usually lasted for only a few seconds. These convulsive seizures would return many times until gradually they gave way to a progressive paralysis of the legs and the animal was no longer able to walk or stand. This condition was soon followed by death. In other cases the paralysis first revealed itself by slight weakness. The animal tired easily and refused to walk. This was usually followed in a day or two by incoördination of the gait. Often spastic contraction of certain muscles of the hindlegs resulted in a very typical spastic gait, simulating the "steppage" gait of multiple neuritis in the human. In some cats the back showed a persistent arching, resembling the position caused by fright. Anaesthesia was rarely present, except in the very advanced stages of the disease. The animals at times seemed to be in pain, as evidenced by the plaintive cries characteristic of cats.

The reflex excitability was often exaggerated. Stimulation of the cut vagus or sciatic caused qualitatively normal reactions (see fig. 2) even in animals with far advanced paralysis. Electric stimulation of the cervical sympathetic caused prompt dilatation of the pupil.

The respiration was usually slowed and very often was irregular (see fig. 1).

The pulse was considerably accelerated during the acute stage; in some cases so rapid that it could not be counted. Irregularities were frequent. Slight muscular exertion, as walking up stairs, caused tachycardia. The blood pressure was practically normal. The pupils were often enlarged and reacted sluggishly to light.

The urine was greatly diminished in volume and gave a strong test for indican, a trace of coagulable protein and bile pigments. Acetone, diacetic acid and sugar were absent. Casts were present in small numbers.

There was a conspicuous absence of oedema in our animals, a symptom which is characteristic of so-called wet beri beri in the human.

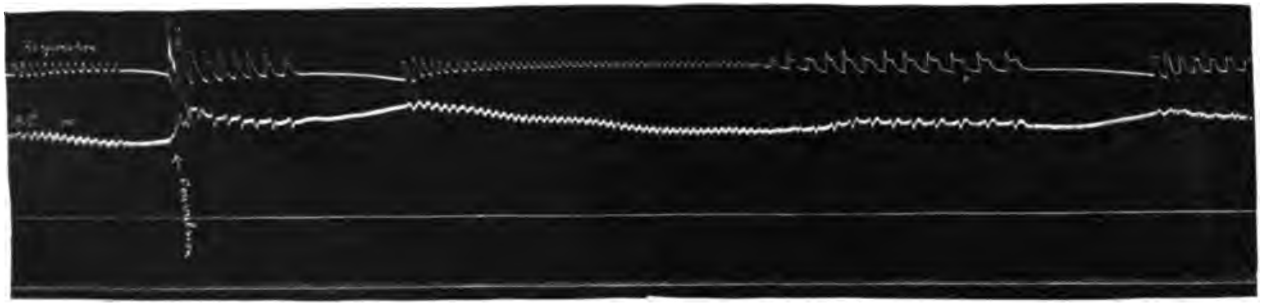


Fig. 1. Cat 47. Light chloretone anaesthesia. Respiratory tracing obtained from pleural cannula. Upstroke indicates inspiration. Blood pressure measured in carotid artery. This tracing shows the marked irregularities in the respiration of a cat with severe polyneuritis. The fluctuations in the blood pressure follow very closely the changes in respiration. Note effect of a convulsion on blood pressure and respiration. The respiration is temporarily very much increased and the blood pressure raised. A short period of apnoea precedes the convulsion.

The disease never caused fever; on the contrary the body temperature fell considerably toward the end. Even in the earlier stages of the disease the heat regulation was upset, as evidenced by a subnormal body temperature resulting from exposure of the animal to a cold room in the winter time. We were unable to explain this disturbance of heat regulation; it may possibly have its origin in an alteration of the nervous mechanism which controls the body temperature.

Death usually occurred several days after the first appearance of the paralytic symptoms. Exceptionally, animals might die during the night, although no definite paralytic symptoms were observed during the previous days.

Pathology. Gross. The gross changes were not very marked. There may be mentioned: fatty appearance of the liver, congestion of the kidney, inflammation of the upper portion of the intestine, dilatation of the heart and occasionally an increase in the pericardial fluid. With the exception of two of the dogs, no evidence of scurvy was found in any of the animals.

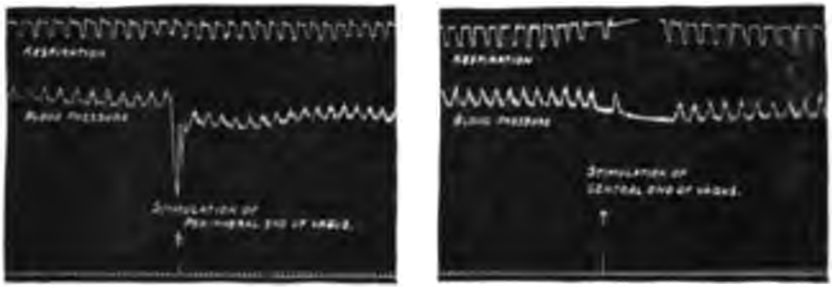


Fig. 2. Dog (no. 7) with severe polyneuritis. Shows effect of stimulation of central and peripheral end of cut vagus. Note normal character of the reaction.

Histological. In almost every instance the sciatic nerves were examined by the Marchi method for evidence of myelin degeneration. In a considerable number the vagi were also examined. Fairly complete histological examinations were made of practically all the important organs of several of the cats. Most of the tissues were fixed with formalin, embedded in colloidin and stained with haematoxylin and eosin. Sections were made from several levels of the spinal cord which were treated by the Marchi method, embedded in paraffin and mounted in chloroform balsam.

The most striking changes were found in the nervous system. The teased sciatic nerves of practically all the animals which developed symptoms, showed myelin degeneration varying from a mild type, characterized by a foamy appearance of the myelin and a swelling at the nodes of Ranvier, to a typical, well-marked degenerative process, involving many fibers, similar to that seen in pigeons with polyneuritis gallinarium. The few spinal cords studied showed some degenerated fibers at all levels, the changes being quite similar to those described by Vedder and Clark ('12) in the case of pigeons. As to location they were somewhat scattered, being found in the posterior, lateral and anterior columns. The amount of change found in the nerves and cord did not always correspond to the severity of the symptoms in the animals.

The changes found in the parenchymatous organs may be summarized as follows:

Heart: The transverse striations are for the most part absent, or only faintly defined. There is considerable fragmentation of the sarcoplasm. It is difficult to determine whether this is due to agonal or post-mortem changes, or to those due to fixing, but it seems that a more or less acute parenchymatous degenerative process must have been present. Fatty degeneration was present only to a slight degree.

Lungs: Endarteritis is quite marked in several cases. The interalveolar septa are thickened. The capillaries are thickened. The capillaries are distended and phagocytic cells are quite numerous in the septa and to some extent in the alveolar lumina. Pigmentation is not marked.

Small intestine: The mucosa shows quite extensive changes. The villi are largely gone. Some round-celled infiltration is present in the base of the mucosa and extends into the submucosa.

Liver: The central vein and its capillaries are distended with blood. The liver cells show numerous small vacuoles, as well as a few large ones, and, besides, a highly granular condition of the cytoplasm.

Pancreas: No definite changes were found with the method of staining employed.

Spleen: There is a marked increase in the connective tissue elements in some cases amounting almost to a true interstitial splenitis. There is a slight amount of colloid degeneration. There are more blood cells in the Malpighian corpuscles than normal and pigmented tissue cells are numerous.

Kidney: The capsule shows no thickening. The glomeruli are for the most part nearly normal, except that the capillaries are somewhat distended. A few of the glomeruli are shrunken and show colloid degeneration, with some round celled infiltration in the vicinity. There are a few small capillary haemorrhages. The epithelial cells of the

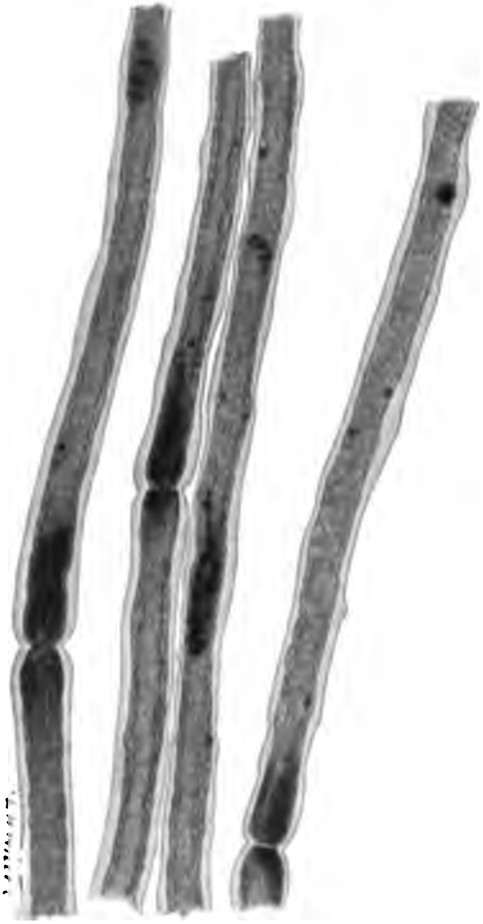


Fig 3

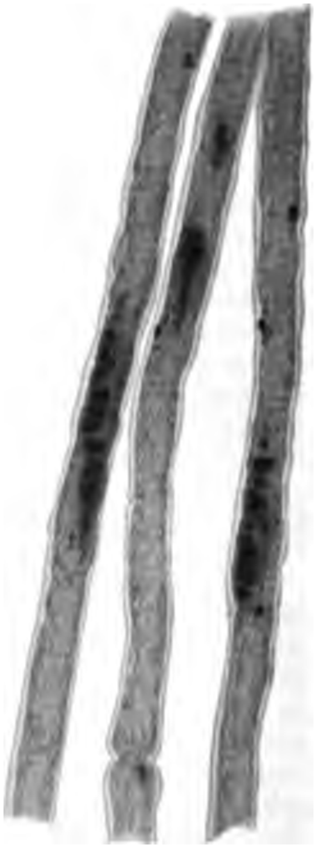


Fig. 4

Fig. 3. Cat 22. Sciatic nerve, teased preparation, Marchi method. Shows marked myelin degeneration. See chart 2.

Fig. 4. Cat 53. Sciatic nerve, teased preparation, Marchi method. Shows fairly well marked myelin degeneration

convoluted tubules show considerable vacuolization and more than the usual amount of granulation of the cytoplasm. The nuclei, for the most part, stain fairly well. In some instances the lumina of the tubules are completely obliterated, the cytoplasm of the cells forming a more or less homogeneous mass of granular material, with the poorly staining nuclei scattered about.

These pathological changes are somewhat similar to, though less extensive than those described by Sundwall ('17) in connection with studies in tissue alterations in malnutrition and in pellagra.

Treatment. When the disease had not advanced too far, the animals could be relieved of their symptoms by means of an active preparation of the antineuritic substance. As will be seen from the protocols, the administration of a single dose of autolyzed yeast filtrate to an animal with severe paralysis caused complete recovery, often within twelve hours. The same result was obtained also after the administration of a single dose of a purified yeast preparation, which had been freed from amino acids, purine bases and other impurities (yeast vitamine 123, chart 6). The improvement, following the administration of antineuritic preparations to severely paralyzed cats, was just as striking as that obtained in pigeons. There was prompt disappearance of all the clinical signs such as paralysis, loss of appetite, high and irregular pulse rate, labored respiration, etc. If the treatment was not continued the symptoms returned after a few days. The continuous daily administration of even a very small dose of autolyzed yeast filtrate resulted in the recovery of lost body weight and prevented the reappearance of polyneuritis.

General considerations. Numerous writers have called attention to the fact that beri beri in the human being is associated with a diet rich in carbohydrates. The disease is especially prevalent among eastern peoples who live on a more or less exclusive diet of white rice. The observations of Little concerning beri beri in Newfoundland seem also to confirm this view, as he attributes the disease to a diet composed of white bread, molasses and fish. Funk ('14) on the basis of feeding experiments on pigeons, tried to establish a relation between carbohydrate metabolism and the antineuritic substance. This author believes that the antineuritic vitamine is in some way concerned with the metabolism of carbohydrates, as he found that an increase in the carbohydrate component of the diet caused a more rapid appearance of avian polyneuritis. This conclusion is also confirmed by the extensive experiments of Braddon and Cooper ('14) who state that the amount of antineuritic substance required by the organism increases with the quantity of carbohydrate metabolized. For the maintenance of health the intake of active substance must, therefore, be adjusted so as to stand in some quantitative relation to the amount of carbohydrate included in the diet.

On superficial consideration, these statements seem to be contradicted by the fact that the present authors were able to induce typical

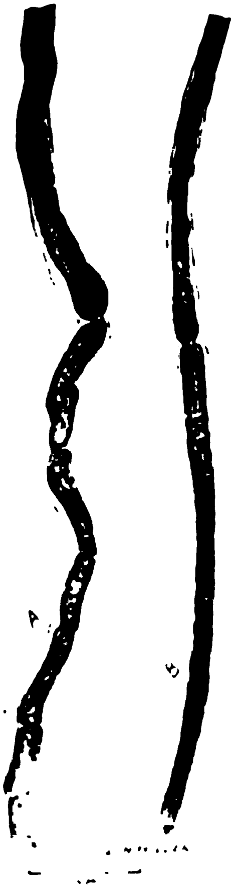


Fig. 5

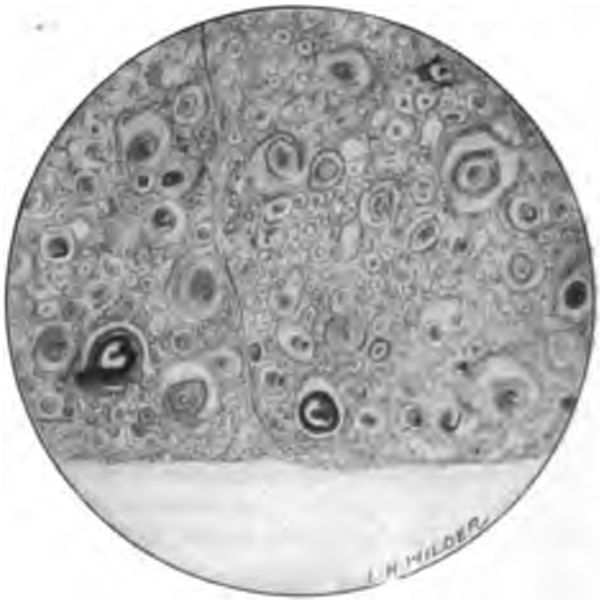


Fig. 6

Fig. 5. Cat 51. Sciatic nerve, teased preparation, Marchi method. *A*, shows early stage of myelin degeneration (foamy appearance). *B*, similar preparation from normal cat.

Fig 6 Cat 4. Cross section of spinal cord (lumbar region), lateral column; Marchi method. Shows degenerated fibers.

polyneuritis by means of an exclusive meat diet, a food which is rich in protein and very poor in carbohydrate. It should be remembered, however, that the intermediate metabolism of protein may lead to the formation of large amounts of carbohydrate. For instance, Reilly Nolan and Lusk ('98) fed a dog suffering from phlorizin diabetes 500 grams of meat and calculated that the sugar production from meat amounted to 58 per cent by weight of the meat protein metabolized. Our observations, therefore, merely supplement the view, expressed by Funk, Braddon and Cooper, that a high protein diet may also lead to an increase in carbohydrate metabolism which may require the presence of a minimum quantity of antineuritic substance.

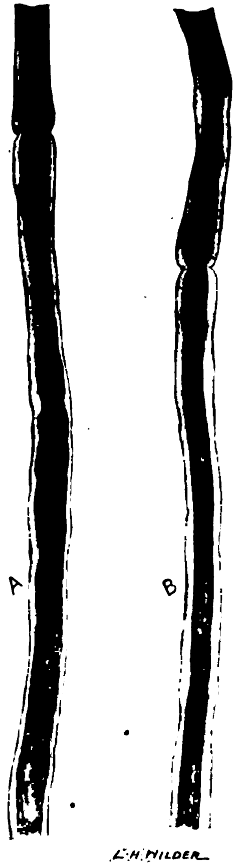


Fig. 7. Dog. 6. *A*, sciatic nerve, teased preparation, Marchi method, showing myelin degeneration. *B*, fiber from sciatic of normal dog.

From the standpoint of dietetics, the results of this investigation emphasize the great stability of the antineuritic substance of meat after a relatively long exposure of this food to high temperatures. Beef can be heated for three hours to 120°C. without its completely losing its antineuritic properties (see chart 1). It is probable that the acid products (lactic acid) of beef are partly responsible for this great heat resistance of the antineuritic substance, as it was shown in this laboratory and elsewhere that an acid reaction tends to preserve its physiological activity. In the preparation of beef for human consumption, this food is rarely exposed throughout to a temperature exceeding 100°C. On the other hand, this research demonstrates that a combination of high temperature and an alkaline reaction leads to the complete destruction of the antineuritic properties of beef. Even in the presence of an alkaline reaction, it is necessary to heat the meat for a considerable time at a temperature above 100°C. Short exposure to 100°C. does not seem to bring about a loss of antineuritic power, as evidenced by some negative experiments not reported in this paper.

In conclusion, it should be pointed out that the present tendency toward alterations of the dietary, as brought about by the war, may very well lead to the occurrence of isolated cases of deficiency diseases in this country and abroad. It is very likely that the early stages of beri beri might easily escape recognition, as the symptoms are not specific for this disease only. This statement is supported by the fact that the prevalence of beri beri among the fishermen of Newfoundland had evidently escaped recognition until Little published his report describing the widespread existence of beri beri in this locality. The history of pellagra in the United States also furnishes evidence to the effect that an unfamiliar disease may exist for years before its presence is recognized.

CONCLUSIONS

1. Polyneuritis has been produced in cats and dogs as the result of an exclusive dietary of lean beef which was heated for three hours at 120°C. in the presence of alkali (sodium carbonate). Proof of this statement is furnished by the symptomatology, treatment and pathology of the disease noted, which are essentially those characteristic of beri beri.

a. Symptoms: The following symptoms were observed in these animals: Diminution of appetite, constipation, loss of body weight, weakness and sometimes drowsiness, followed by paralytic symptoms, tonic convulsions, spasticity of certain groups of muscles and disturbances of the circulation and respiration.

b. Treatment: The oral administration of active preparations of the antineuritic substance of yeast to paralyzed animals is followed promptly by the disappearance of the symptoms; and the continued administration of these preparations prevents the recurrence of the disease.

c. Pathological changes: Certain histopathological changes especially the changes involving the nervous system are described. Animals showing severe paralysis exhibit no qualitative changes in the reaction of various nerves to electric stimulation.

2. The disease is due to a deficiency of the diet in antineuritic substance and not to a deficiency in the other essential dietary components (amino acids, fat-soluble vitamine, etc.).

3. Exposure of the beef for three hours to a temperature of 120°C., without the previous addition of alkali, does not completely destroy the antineuritic power of this food. It is therefore concluded that the ordinary preparation of meat for human consumption does not lessen its food value in this respect.

4. The various species of animals show a considerable difference in their susceptibility to polyneuritis, as evidenced by the different length of time which is necessary to induce the disease by the same deficient diet. Cats respond to the deficient diet with the greatest regularity and are, therefore, best adapted for physiological studies of the function of the antineuritic substance.

BIBLIOGRAPHY

Andrews, V. L. '12. Philippine Journ. Sci., vii, 80.
Braddon, W. L. '07. The cause and prevention of beriberi. London.
Braddon and Cooper. '14. Journ. Hyg., xiv, 351.
Fraser and Stanton. '09. Studies from the Inst. for Med. Research, Federated Malay States, no. 10. Also see Lancet, 1909, 451.
Funk C. '14. Zeitschr. f. physiol. Chem., lxxxix, 378.
Hose, C. '05. Brit. Med. Journ., ii, 1098.
de Lacerda, J. B. '85. Berl. klin. Wochenschr., xxiii, 159.
Little. '12. Journ. Amer. Med. Assoc., lviii, 2029.
'14. Ibid., lxiii, 1287.
Reilly, Nolan and Lusk. '98. This Journal, i, 395.
Rommel and Vedder. '15. Journ. Agric. Res., v, 489.
Schaumann. '10. Arch. f. Schiffs. u. Trop.-Hyg., xvi, Beiheft 8.
Shiga and Kusama. '11. Arch. f. Schiffs. u. Trop.-Hyg., xv, 59.
Sundwall. '17. Hyg. Lab. Bull., no. 106.
Vedder and Clark. '12. Philippine Journ. Sci., vii, 423.
Voegtlin, Sullivan and Myers. '16. Public Health Repts., xxxi, 935.
Watson and Hunter. '06. Journ. Physiol., xxxiv, 111.

INDEX TO VOLUME XLVII

ACACIA method for determining blood volume, 302.

Adrenalin, effect of, on blood pressure, 286.

——, —— ——, —— muscular fatigue, 178, 185.

Aerotonometer, a new form of, 137.

——, the lung as an, 143.

Alkali administration and lactic acid in urine, 189.

Alvarez, W. C. and E. Starkweather.
  XIV. Differences in the catalase content of muscle from different parts of the stomach, 60.
  XV. The catalase content of the mucous membrane from different parts of the digestive tract, 67.
  XVII. The metabolic gradient underlying colonic peristalsis, 293.

Amino-acids, effect of, on catalase, 351.

Anderson, C. E. See Riddle and Anderson, 92.

Atwell, W. J. and C. J. Marinus. A comparison of the activity of extracts of the pars tuberalis with extracts of other regions of the ox pituitary, 76.

Auer, J. and S. J. Meltzer. The blood-pressure curve following an intraspinal injection of adrenalin, 286.

BLOOD coagulation: heparin and pro-antithrombin, 328.

—— count during digestion, 399.

——, effect of secretin injections on, 342.

——, factors controlling volume-flow of, 438.

Blood, method of determining volume-flow of, 428.

—— pressure, effect of adrenalin on, 286.

—— serum proteins, regeneration of, 356, 370, 379.

—— volume by acacia method, 302.

Brain of normal and ataxic pigeons, chemistry of, 124.

Buckner, G. D., R. H. Wilkins and J. H. Kastle. The normal growth of White Leghorn chickens, 393.

Burge, W. E. The influence of the ingestion of the amino-acids, adequate and inadequate proteins upon the production of catalase, 351.

—— and A. J. Neill. Further study on the effect of food in increasing oxidation, 13.

Burton-Opitz, R. and R. Dinegar. The viscosity of urine, 220.

CARBON dioxide, undissociated, significance of, in respiration, 43.

Catalase content of gastric muscle, 60.

—— —— —— mucous membrane of digestive tract, 67.

——, effect of amino-acids and proteins on, 351.

Cerebral cortex, chemical stimulation of, 283.

Chickens, normal growth of, 393.

Clark, J. H. The photo-chemical effect of certain fluorescent substances on rennin, 251.

DIET, influence of, on protein regeneration, 370.

Digestion, blood count during, 399.

Digestive tract, catalase content of mucous membrane of, 67.

Dinegar, R. See Burton-Opitz and Dinegar, 220.
Downs, A. W. and N. B. Eddy. Secretin. IV. The number of red and white corpuscles in the circulating blood during digestion, 399.

ECK fistula and plasma depletion, effect of, on protein regeneration, 379.
Eddy, N. B. See Downs and Eddy, 399.
Egg yolk and egg albumen, effects of quinine on production of, 92.
Electrical deflections, 411.

FASTING, influence of, on protein regeneration, 356.
Fatigue, muscular, effect of adrenalin on, 178, 185.
Fluorescent substances, photo-chemical effect of, on rennin, 251.
Foster, J. P. See Mann and Foster, 278.
Fujimoto, B. The influence of secretin on the number of red and white corpuscles and the ferments and sugar content of the blood, 342.

GASSER, H. S. See Meek and Gasser, 302.
Gastric muscle, catalase content of, 60.
Gesell, R. Studies on the submaxillary gland:
- I. Electrical deflections in general, 411.
- II An automatic and bloodless method of recording the volume-flow of blood, 428.
- III Some factors controlling the volume-flow of blood, 438.
- IV A comparison of the effects of hemorrhage and of tissue-abuse in relation to secondary shock, 468
- V An automatically filling and recording spirometer, 507.

Gillett, L. H., L. Wheeler and A. B. Yates. Material lost in menstruation of healthy women, 25.
Growth, normal, of chickens, 393.
Gruber, C. M. IX. The antagonistic action of epinephrin to the fatigue produced by the perfusion of acid sodium phosphate, 185.
—— and O. S. Kretschmer. VIII. The effect of adrenalin upon the fatigue produced by the injection of the fatigue products, lactic acid and acid potassium phosphate, 178.

HEART, beating, heat liberated by, 156.
Heat liberated by beating heart, 156.
Hemorrhage, effects of, on blood flow, 468.
Heparin and blood coagulation, 328.
Holt, E. See Howell and Holt, 328.
Howell, W. H. and E. Holt. Two new factors in blood coagulation—heparin and pro-antithrombin, 328.
Hurwitz, S. H. See Kerr, Hurwitz and Whipple, 356, 370, 379.

IRWIN, M. The nature of sensory stimulation by salts, 265.

KASTLE, J. H. See Buckner, Wilkins and Kastle, 393.
Kerr, W. J., S. H. Hurwitz and G. H. Whipple. Regeneration of blood serum proteins:
- I. Influence of fasting upon curve of protein regeneration following plasma depletion, 356.
- II. Influence of diet upon curve of protein regeneration following plasma depletion, 370.
- III. Liver injury alone: liver injury and plasma depletion: the Eck fistula combined with plasma depletion, 379.

Kidney, action of pituitary extract on, 1.

King, J. L. Possible periodic variations in the extent of the knee-jerk in women, 404.
Knapp, H. J. See Macleod and Knapp, 189.
Knee-jerk in women, periodic variations of, 404.
Knowlton, F. P. and A. C. Silverman. The action of pituitary extract on the kidney, 1.
Koch, M. L. and O. Riddle. The chemical composition of the brain of normal and ataxic (?) pigeons, 124.
Kretschmer, O. S. See Gruber and Kretschmer, 178.

Lactic acid, alkali administration and, in urine, 189.
Lake, G. C. See Voegtlin and Lake, 558.
La Mer, V. K. See Riddle and La Mer, 103.
Livery injury, effect of, on protein regeneration, 379.
——, secretory pressure of, 278.
Luminescence of Watasenia scintillans (Berry), 534.
Lund, E. J. Quantitative studies on intracellular respiration:
  III. Relation of the state of nutrition of Paramecium to the rate of intracellular oxidation, 167.
  IV. The rates of CO_2 production by starved and fed Paramecia, and their possible relation to the oxidation in the unfertilized and fertilized sea urchin's egg, 318.
Lung as an aerotonometer, 143.

Macleod, J. J. R. and H. J. Knapp. The influence of alkali administration on the urinary excretion of lactic acid, and the possible significance of the latter in maintaining neutrality in the body, 189.
Mann, F. C. Studies on experimental surgical shock, 231.
Mann, F. C. and J. P. Foster. The secretory pressure of the liver with special reference to the presence or absence of a gall bladder, 278.
Marinus, C. J. See Atwell and Marinus, 76.
Martin, E. G. and W. H. Rich. Muscular strength and muscular symmetry in human beings. II. In adult males, 29.
Maxwell, S. S. Chemical stimulation of the cerebral cortex, 283.
Meek, W. J. and H. S. Gasser. Blood volume. A method for its determination with data for dogs, cats and rabbits, 302.
Meltzer, S. J. See Auer and Meltzer, 286.
Menstruation of healthy women, material lost in, 25.
Mucous membrane of digestive tract. catalase content of, 67.
Muscle, gastric, catalase content of, 60.
Muscular strength and muscular symmetry in adult males, 29.

Neill, A. J. See Burge and Neill, 13.
Nerve, irritability of, effect of osmotic pressure on, 512.
Nervous reflexes in traumatic shock, 208.

Osmotic pressure, effect of, on irritability of nerve, 512.
O'Sullivan, P. M. A new form of aerotonometer, 137.
——. The lung as an aerotonometer, 143.
Oxidation, effect of food in increasing, 13.

Paramecium, intracellular respiration in, 167, 318.
Peristalsis, colonic, metabolic gradient of, 293.
Photo-chemical effect of fluorescent substances on rennin, 251.
Pigment, post-mortem, in pigmentless retinas and choroids, 103.

Physiol

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

THE ACTION OF PITUITARY EXTRACT ON THE KIDNEY. *F. P. Knowlton and A. C. Silverman.* 1

FURTHER STUDY ON THE EFFECT OF FOOD IN INCREASING OXIDATION. *W. E. Burge and A. J. Neill* 13

MATERIAL LOST IN MENSTRUATION OF HEALTHY WOMEN. *L. H. Gillett, L. Wheeler and A. B. Yates* 25

MUSCULAR STRENGTH AND MUSCULAR SYMMETRY IN HUMAN BEINGS. II. IN ADULT MALES. *E. G. Martin and W. H. Rich* 29

THE SIGNIFICANCE OF UNDISSOCIATED CARBON DIOXIDE IN RESPIRATION. *R. W. Scott* .. 43

XIV. DIFFERENCES IN THE CATALASE CONTENT OF MUSCLE FROM DIFFERENT PARTS OF THE STOMACH. *Walter C. Alvarez and Esther Starkweather* 60

XV. THE CATALASE CONTENT OF THE MUCOUS MEMBRANE FROM DIFFERENT PARTS OF THE DIGESTIVE TRACT. *Walter C. Alvarez and Esther Starkweather* 67

A COMPARISON OF THE ACTIVITY OF EXTRACTS OF THE PARS TUBERALIS WITH EXTRACTS OF OTHER REGIONS OF THE OX PITUITARY. *Wayne J. Atwell and Carleton J. Marinus.* 76

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN BIRDS. VIII. THE EFFECTS OF QUININE ON THE PRODUCTION OF EGG YOLK AND EGG ALBUMEN. *Oscar Riddle and Carl E. Anderson.* 92

POST-MORTEM MELANIN PIGMENT FORMATION IN PIGMENTLESS RETINAS AND CHOROIDS OF WHITE RING-DOVES. *Oscar Riddle and Victor K. La Mer* 103

THE CHEMICAL COMPOSITION OF THE BRAIN OF NORMAL AND ATAXIC (?) PIGEONS. *Mathilda L. Koch and Oscar Riddle* 124

A NEW FORM OF AEROTONOMETER. *Paul M. O'Sullivan* 137

THE LUNG AS AN AEROTONOMETER. *Paul M. O'Sullivan* 143

ON THE HEAT LIBERATED BY THE BEATING HEART. SECOND COMMUNICATION. *Charles D. Snyder* 156

VOL. XLVII—No. 1
Issued September 1, 1918

BALTIMORE, U. S. A.
1918

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

THE AMERICAN JOURNAL OF PHYSIOLOGY is issued monthly by the American Physiological Society under the direction of the Council of the Society. From two to three volumes each of about six hundred pages are published yearly. The subscription price per volume in the United States and Canada is $5.00; in other countries $5.25.

Manuscripts submitted for publication may be sent to any member of the Council or to the Managing Editor, Dr. D. R. Hooker, Johns Hopkins Medical School, Washington and Monument Streets, Baltimore, Md. They should be packed flat, carefully protected from injury and insured.

Each article should conclude with a brief summary of results suited to the needs of reference journals.

References to literature cited should contain: first the name of the author or authors; second the name of the journal or book; third the year; fourth the volume number and fifth the page. These references should be collected on a separate page at the end of the article with corresponding bracketed numbers in the text. Foot notes are to be avoided whenever possible.

All figures for illustration should be submitted in such form as to admit of photographic reproduction without retouching or redrawing. Marginal letters to figures cannot be set in type and should, therefore, be written in India ink and regarded as a part of the figure. When artist's work is required to make figures suitable for reproduction this will be charged to authors. Unless specific instructions are given by authors the printer will be allowed to use his judgment in determining the amount of reduction on all figures. In any case the plates should never exceed the dimensions of the printed matter of the JOURNAL page unless the author is prepared to pay the cost of an insert page.

Tracings on smoked paper reproduce well if the white lines are distinct and separate and the background uniform black. Authors may safely retouch breaks or weak spots in the white lines with white ink and fill in the background with India ink. Curves laid on coordinate paper should be in India ink. Background lines in blue will not reproduce; red, yellow and green will reproduce black. Plotted curves and drawings yield better results if the originals are large enough to permit of considerable reduction in the plates used for illustration, but authors are warned to make due allowance for this in the breadth of lines and size of lettering. Particular letters and numbers to paste on tracings and figures will be furnished upon request if the amount of reduction to be used is stated.

YOU

MAY

HAVE

A

CATALOG

Acidosis Testing Outfits

Kidney Functionation Test

Urea Estimating Reagent

Blood Collecting Devices

Bacterial and Biochemic Therapeutic Agents

Illustrated and Described

Send request to

HYNSON, WESTCOTT AND DUNNING

BALTIMORE **MARYLAND**

Bacteriologic Peptone

PARKE, DAVIS & CO.

CHEMICALLY ANALYZED

CONTAINS:

Total Nitrogen	not less than 14.0%
Mineral matter	not more than 3.5%
Moisture	not more than 6.0%

Used in bouillon according to standard methods.

FURNISHES:

Diphtheria Toxin .	of which 0.25 mil = 1 L+ Dose
Tetanus Toxin . .	of which 0.001 mil = 1 M. F. D.

BACTERIOLOGICALLY TESTED

Bacteriologic Peptone, P. D. & Co., is the one American-made peptone which fulfills the varied and exacting requirements of all bacteriologic work.

It has a maximum content of the proper amino acids, or protein "building stones."

It has the proper chemical structure for the production of diphtheria and tetanus toxins of highest potency.

Its efficiency has been established under practical working conditions by many of the best known scientists in North America. This work included, in addition to the routine of the bacteriological laboratory, such special procedures as cultivation of spirochætæ; analysis of milk, water and soil; standardization of disinfectants, manufacture of serums and vaccines, indol production tests, and some of the other more delicate biologic reactions.

Bacteriologic Peptone, P. D. & Co., is readily and completely soluble in all proportions, furnishing clear bouillon and agar.

Supplied in 1-pound bottles and 5- and 10-pound cans.

WRITE FOR LITERATURE AND QUOTATIONS.

Home Offices and Laboratories,
Detroit, Michigan.

Parke, Davis & Co.

Physiol
Stanford University Library
FEB 4 1919

THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

STUDIES ON THE SUBMAXILLARY GLAND. I. ELECTRICAL DEFLECTIONS IN GENERAL. *Robert Gesell* 411

STUDIES ON THE SUBMAXILLARY GLAND. II. AN AUTOMATIC AND BLOODLESS METHOD OF RECORDING THE VOLUME-FLOW OF BLOOD. *Robert Gesell* 428

STUDIES ON THE SUBMAXILLARY GLAND. III. SOME FACTORS CONTROLLING THE VOLUME-FLOW OF BLOOD. *Robert Gesell* 438

STUDIES ON THE SUBMAXILLARY GLAND. IV. A COMPARISON OF THE EFFECTS OF HEMORRHAGE AND OF TISSUE-ABUSE IN RELATION TO SECONDARY SHOCK. *Robert Gesell* 468

STUDIES ON THE SUBMAXILLARY GLAND. V. AN AUTOMATICALLY FILLING AND RECORDING SPIROMETER. *Robert Gesell* 507

THE EFFECT OF OSMOTIC PRESSURE ON THE EXCITABILITY OF THE NERVE. *Rinnosuke Shōji* 512

A PHYSIOLOGICAL STUDY ON THE LUMINESCENCE OF WATASENIA SCINTILLANS (BERRY). *Rinnosuke Shōji* 534

EXPERIMENTAL MAMMALIAN POLYNEURITIS PRODUCED BY A DEFICIENT DIET. *Carl Voegtlin and G. C. Lake* 558

INDEX 591

VOL. XLVII—No. 4
Issued January 1, 1919

BALTIMORE, U. S. A.
1919

Entered as second class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailin
at special rate of postage provided for in Section 1103, Act of October 3, 1917. Authorized on July 5, 1918.

STANFORD UNIVERSITY LIBRARY

To avoid fine, this book should be returned on or before the date last stamped below

LIBRARY OF THE
SCHOOL OF BIOLOGY